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AN EXPERIMENTAL STUDY

OF

AVIAN BERIBERI.

PART I. INTRODUCTION.

PART II. AVIAN BERIBERI.

PART III. BIOCHEMICAL EXPERIMENTS.

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AN EXPERIMENTAL STUDY  
OF  
AVIAN BERIBERI.

"Aves quasdam rerum augurandarum causa natas esse  
putamus".

Cicero.

PART 1.  
INTRODUCTION

The years of Warfare have had a deep effect on Medicine, for in every department advances have been made, - in preventive Medicine, in Surgery, in Bacteriology, in Psychiatry, to mention but a few; nowhere, however, has the advance been more striking than in our knowledge of Nutrition. The importance of the food supply was realised at an early date by all the combatants; the whole subject of Nutrition was intensively studied and as a result our previously accepted theories have undergone almost complete revision. It has been conclusively shown that if normal metabolism is to be maintained there must be present in the diet certain special substances, in addition to the well-known food stuffs, proteins, carbohydrates, fats, salts, oxygen and water. These additional substances were first named "Vitamines", but are now more commonly known as "Accessory food factors", a term which, though implying little, at least does not lead to any erroneous conclusion as to their chemical composition or mode of action. So far the existence of three accessory food factors has been definitely established - Fat Soluble A, Water Soluble B and the Anti-Scurvy or Water Soluble C factor. The absence of Fat Soluble A factor from the diet of experimental animals is followed by progressive asthenia and an especial liability to broncho-pneumonia, while there occurs in the eye a condition known as Xerophthalmia, which is regarded by many authorities as almost pathognomonic. Possibly the Anti-rachitic factor on which Mellanby (1) is working is identical with Fat Soluble A. Water Soluble B factor on the other hand would appear to be the same as the Anti-neuritic Vitamine isolated by Funk (2) in 1911; one function is associated with the prevention of Beriberi; but it has another of perhaps even greater importance, for in conjunction with/



with Fat Soluble A it is essential to the growth of young animals.

Although a very large amount of work has been carried out on the Accessory food factors, we are still entirely ignorant of their chemical composition and mode of action. The object of the following research ~~then~~ has been to assist in elucidating the second problem, in so far as Water Soluble B factor is concerned, while an attempt has also been made to throw light on the numerous changes which occur in the body as a result of the absence, partial or complete, of this factor from the diet.

As is well known, the complete deprivation of Water Soluble B factor leads in man to the occurrence of the disease known as Beriberi. Beriberi itself is rarely seen except in Eastern countries, where it still forms a serious menace to the population. Thus in 1918 there were nearly 16,000 cases of Beriberi in Hong-kong; in The Russo-Japanese War there were more than 200,000 cases in the Japanese Army, while in the present war outbreaks were reported from France, Gallipoli and Mesopotamia. It is of interest to note that whereas Scurvy has almost disappeared from the Navy, Beriberi is still comparatively common. During three and a half years in the East, I encountered cases from no less than sixteen of H.M. Ships stationed in the Persian Gulf and Red Sea. A severe outbreak of Beriberi was also the direct cause of the untimely end of one of Germany's auxiliary cruisers.

Thus though Beriberi itself is largely confined to Eastern populations whose staple diet is polished rice, or to periods of stress and strain when food supplies run short, it must be remembered that the disease known as Beriberi merely represents the last stage in a long pathological process, set up by the absence of Water Soluble B factor. In the main the clinical symptoms constituting Beriberi are all referable to the nervous system and this system is the last to suffer from the lack of Water Soluble B factor, since it is protected at the expense of the other organs of the body.

In diets, therefore, which, though not wholly, are partially lacking in Water Soluble B factor pathological changes are liable to occur in almost any organ of the body. A deficiency in Accessory food factors may thus be an etiological factor in a very large number of diseases. It may be the cause of sterility in the male or female, it may explain why the baby does not thrive on its mother's milk or why it fails to grow on patent foods. It may determine the onset of bacterial infection, or by its action on the alimentary canal be a factor in producing intestinal disease.

Many mental and nervous conditions may be associated with an abnormal metabolism of Accessory factors, while an excess of these same factors by their stimulating action on cell growth may even be an accessory cause of malignant disease.

The/



The subject of Accessory food factors is as wide as Medicine itself, but though the possibilities of the subject may be dimly viewed, precise knowledge is lacking. Precision can alone be obtained by patient research.

Fortunately for the purposes of research, it has been found that when fed on a diet of polished rice, certain birds, such as fowls and pigeons, develop symptoms of Beriberi with regularity and rapidity.

This important discovery is due to Eijkmann (3), a Dutch physician, who in 1890, while working in Batavia found that certain of the fowls confined in his laboratory were showing signs of paralysis. Their symptoms in many ways resembled those of human beings with Beriberi.

On investigation, it was found that only those fowls developed paralysis which had been fed exclusively on polished rice, others which had received kitchen scraps or foraged for their food remained healthy. After a long series of experiments, it was conclusively shown that paralytic symptoms were not caused by an infectious agent, but were due solely to some difference in property between polished and unpolished rice, since fowls were protected when fed on unpolished rice or rice polishings, but inevitably developed paralysis when fed on polished rice alone. Eijkmann was inclined to believe that the cause of "polyneuritis avium", as the disease was called, is a toxin produced by the fermentation of polished rice in the crop, an antidote to this hypothetical toxin being found in rice polishings. His conclusions were ably stated in a series of publications from 1890-96, but unfortunately were received with much criticism and scant attention. It was not until the experiments with polished rice were repeated in full by Grijns (4) in 1900 and Hulshoff Pol (5) in 1909 that any serious attention was paid to avian polyneuritis, and even then, so dominating was the idea that parasite and toxin constituted the sole "materies morbi" that the true etiology was entirely obscured. At length after Braddon (6) and Fraser and Stanton (7) had shown the close connection between polished rice and Beriberi and Funk had "isolated" his Anti-neuritic Vitamine from rice polishings, a more correct idea of the real cause of Polyneuritis avium gained ground.

Since then the disease has been closely investigated by a number of observers - Schaumann (8), Shiga and Kusama (9) Chamberlain, Vedder and Williams (10), Funk and Douglas (11) all of whom have added materially to our knowledge.

At first sight it might almost seem superfluous to direct further attention to so well worked a subject were it not that quite recently McCarrison (12) by his experiments on pigeons has shown that there still remain a number of points of interest in regard to the pathology and etiology of Avian Beriberi.

The following study was therefore undertaken in the first place to confirm McCarrison's findings in pigeons and to/

to extend them if possible to the fowl, while secondly it was desired to discover if from the histological examination of the morbid tissues there could be found any clue to the mystery that surrounds the rôle of accessory food factors in normal metabolism.

With this end in view, the first part of this paper deals with the morbid anatomy of the disease, as seen in pigeons and fowls, while the second is concerned with biochemical experiments which tend perhaps to shed a certain light on the part played by at least one of the Accessory food factors in the Vital economy.

In the present paper the term "polyneuritis avium" has been dropped, "Avian Beriberi" being used in its place. The pathological changes associated with an exclusive diet of polished rice affect not only the peripheral nerves, but every organ in the body; furthermore, as symptoms akin to those of Beriberi in man can be produced experimentally not only in birds but in many animals, it seems somewhat superfluous to employ a particular term for Beriberi as it occurs in birds.

Although in feeding experiments, certain conditions can be fixed and their effects noted with a very considerable degree of accuracy, it must be remembered that there are others, inherent in the nature of the experiments themselves, which it is impossible definitely to control. The chief of these latter conditions is to be found in the enforced captivity which is essential if the diet is to be restricted, for unfortunately captivity and even domestication are <sup>liable</sup> apt to have certain effects on the internal organs, and more particularly on the sexual organs, as a result of which their function is apt to be decreased or even totally inhibited.

It must also be remembered in this connection that it would be almost strange if, as the result of feeding experiments, small differences in morbid anatomy were not met with by workers in various countries. In animals suffering from deficiency diseases there are two principle factors which influence the morbid anatomical appearances - the rapidity of onset of the symptoms and the presence of intercurrent infectious agents. The influence of such agents cannot wholly be excluded for although the sterility of the blood and organs may be successfully demonstrated, it is impossible absolutely to discount the effects of gastro-intestinal infections or intestinal parasites. The distribution of the latter especially varies with climate for whereas in the tropics, parasitic worms were met with in the intestine of every pigeon and fowl examined, in Europe they are only rarely encountered. Before discussing the histological changes met with in birds suffering from Beriberi, it will therefore be necessary to outline briefly the conditions under which the experiments were conducted. The feeding experiments on fowls were for the most part/



part conducted during the spring and summer of 1919 at Port Said, Egypt, but it was not until my return to this country that, thanks to a grant from the Carnegie Research Trust, I was enabled to make use of the facilities provided by the Royal College of Physicians' Laboratory, Edinburgh. I desire to express my sincere thanks both to the Carnegie Trust and to the Royal College of Physicians, Edinburgh.

The first series of experiments was conducted on fowls, while later, similar experiments were carried out with pigeons. Although not possible to obtain undomesticated birds, the degree of domestication in Egyptian fowls is not as great as in fowls in this country. All the birds employed were approximately three quarters grown and were roughly about the same weight at the beginning of the experiment. Egyptian fowls never attain the same size as their European relatives. Altogether 43 fowls were employed in this experiment. Of these, four were found to be infected, twelve were fed on mixed grain as controls, fifteen on an exclusive diet of polished rice, while twelve were given water only, in order to compare the effects of complete inanition.

The cages used had not previously been employed for keeping animals. Two cages were used for each bird; the cages were employed on alternate days, the one not in use being thoroughly cleaned and disinfected. The cages were placed in the open air but were protected from the direct rays of the sun. They were so arranged that the birds could not see one another. Although not so necessary in the case of pigeons, separation is essential in the case of fowls, since the young cocks at any rate soon wear themselves out by fighting. The experimental birds were fed on a highly milled Patna rice, throughout the course of the experiment. However highly milled a rice may be, a few grains are almost always found with portions of the aleurone layer and pericarp still adherent. Before use therefore the rice was carefully picked over by hand, and any suspicious looking granules discarded. The rice was then autoclaved for three quarters of an hour at 120°C. Finally a weighed quantity of rice - 70 grammes - roughly one-tenth of the body weight at the beginning of the experiment - was given in two equal portions to each bird night and morning. No attempt was made to feed the animals forcibly, as it was desired to obtain some idea of the amount of rice eaten throughout the course of the experiment. Water was given in unlimited quantities; it was boiled immediately before being placed in the drinking cups. By these precautions secondary infections were to a certain extent excluded. In fowls four cases were encountered out of 43 or 9.3%. One of these cases suffered from coccidiosis of the liver and began to show symptoms of paralysis after  $3\frac{1}{2}$  days on a diet of polished rice. This is the shortest incubation period/

period I have every noted in a case of Avian Beriberi. Two other fowls suffered from infection with *Bacillus pyocyaneus* and one with *B. coli*.

In the case of pigeons similar precautions were observed; 41 pigeons were employed. Of these 15 were fed on the diet of polished rice, while 12 were used as controls. 6 pigeons were given a small ration of Oats and Maize - 10 grammes of each grain on alternate days, with the addition of one-tenth of the body weight of Yeast every third day. Those pigeons which had not already died were killed off on the thirty-fifth day of the experiment, the object being to investigate chronic starvation with a full supply of water Soluble B factor in the diet.

Septicaemic conditions would seem to be rather more common in pigeons than in fowls, for eight out of the forty one pigeons were infected or 19.5%. Of these two were infected with *B. pyocyaneus*, six with *B. coli*.

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## PART 11.

## AVIAN BERIBERI.

1. CLINICAL OBSERVATIONS of
  - (a) Avian Beriberi,
  - (b) Acute & chronic inanition in birds.
2. HISTOLOGICAL OBSERVATIONS
  - (a) in birds
  - (b) in man
  - (c) Beriberi in man and animals compared.
3. PATHOGENESIS - A critical Discussion.

## (1) CLINICAL OBSERVATIONS.

## (a) Avian Beriberi -

(1) THE INCUBATION PERIOD. Although, since Beriberi is not due to a parasitic agency, the use of the term "Incubation period" may not be strictly accurate in this connection, nevertheless it is usually employed to denote the period during which an animal or man can exist on a diet entirely deficient in Water Soluble B factor without showing symptoms referable to this deficiency.

For fowls the period is usually said to be from 20-35 days. Thus Vedder and Clark (1) as the result of their experiments on 124 fowls, found that the average was 26.86 days. In the present instance the average period was 31.5 days for uninfected fowls, the shortest period being 18 days, the longest 74 days. In the case of pigeons the generally accepted period is from 15 to 25 days. McCarrison (2) however found that the period for his pigeons varied from 30-100 days, the average being 67.1 days. In the present experiment, the average was 27.2 days, the shortest 21 days, the longest 43 days.

It has, however, been found that several factors influence the incubation period, the principle being,

- 1) Age.
- 2) Sex.
- 3) Diet prior to the experiment.
- 4) Exertion during the experiment.
- 5) Quantity of Rice eaten during the experiment
- 6) Bacterial or other infection.

1. Age. Other things being equal, this would appear to be the most important factor in determining the rate of onset of symptoms. The identity of Funk's Anti-Neuritic Vitamine with the Water Soluble B factor first described by McCollum and Davis (3) in 1913 would now seem to be universally conceded (McCollum & Kennedy 1916 (4) and Drummond 1917 (5)). Since the presence of Water Soluble B factor is necessary not only for the maintenance of normal nutrition but also for the continuance of growth in the young, it is not surprising to find that in young animals, where there is as it were a two-fold demand for the factor, the symptoms, which occur when it is absent, should appear at an earlier date than in adults.

Drummond (6) has also found that age is the main factor in determining the rapidity of onset of the disease in rats, since "the length of time that a rat is able to maintain itself on a diet deficient in Water Soluble B factor is directly proportional to the age at which the restriction was imposed".

The question of age may thus to a certain extent explain the discordant results obtained by various workers in regard to the incubation period of Avian Beriberi.

2. Sex. In birds which have been allowed to eat naturally the incubation period is slightly shorter in the male than in the female. This is probably due in part to the more active life of the male, in part to his more voracious appetite, since artificial feeding equalises the incubation period in the two sexes. In man, Beriberi is far more common in the male than in the female.

3. Diet prior to the experiment. Very little is at present known in regard to the metabolism of Water Soluble B factor. From the recent experiments of Gaglio (7) it would appear that in man at any rate Water Soluble B factor is excreted in appreciable amount in the Urine, but there is no information as to whether the body can store up a large supply of Water Soluble B factor.

A few experiments were therefore carried out with pigeons. Twelve pigeons were employed, divided into four groups, A, B, C & D:-

Group A was fed for 10 days on  $\frac{1}{20}$  the body weight of polished rice +  $\frac{1}{1000}$  the body weight of Yeast.

Group B was fed for 10 days on  $\frac{1}{20}$  the body weight of polished rice +  $\frac{1}{100}$  the body weight of yeast.

Group C was fed for 10 days on  $\frac{1}{20}$  the body weight of polished rice +  $\frac{1}{10}$  the body weight of yeast.

Group D was fed for 10 days on  $\frac{1}{20}$  the body weight of polished rice +  $\frac{1}{5}$  the body weight of yeast.

After/



After ten days on the above diets the birds were placed on an ad libitum diet of polished rice and the incubation period noted.

The average incubation period for Group A was 22.4 days
" " " " " " B " 31.2 "
" " " " " " C " 29.4 "
" " " " " " D " 30.9 "

It will thus be seen that with the smallest quantity of Yeast there was little or no protection afforded. Group B however showed a definite though slight prolongation of the incubation period, while in Groups C & D the incubation period is almost identical with that in Group B.

In other words, it would seem that the tissues are only able to take up and store a very limited amount of Water Soluble B factor; any excess over this fixed amount being either excreted or destroyed in the tissues.

4. Exertion during the experiment. The effect of exertion in hastening the onset of Beriberi in man has often been noted. Thus in ship Beriberi it has been found that the greater number of cases occurs among stokers whose work is more arduous than that of deckhands. It is of interest to note that recently in another deficiency disease, Pellagra, Boyd (8) has shown that hard work precipitates the onset in those who are in what is termed a "prepellagroid" condition. The effect of exercise in hastening the onset of Beriberi in fowls was illustrated by the following experiment. Three fowls, living under conditions similar to those described above, were kept on the move for one hour a day through the activities of a small boy engaged for the purpose. The incubation period in these three cases was 16, 19 and 21 days respectively, while it is noteworthy that in every case the bird died within 36 hours of the onset of symptoms.

Attempts to show the effects of fatigue on pigeons fed on a polished rice dietary have not been so successful, possibly because, under natural conditions, the pigeon is capable of undertaking long and arduous flights whereas the domestic fowl is not by nature a very active bird.

The effect of fatigue in shortening the incubation period is more particularly interesting when it is remembered that marked chromatolysis is produced in the cells of the central nervous system both by the absence of Water Soluble B factor from the diet and by fatigue.

5. The Quantity of Rice eaten during the experiment. In 1907 Maurer (9) found that when fowls were fed on polished rice, those birds receiving the largest quantity of rice developed symptoms of Beriberi first. Chamberlain/

Chamberlain, Bloombergh and Kilbourne (10) on the other hand found that birds consuming large rations of rice remained freer than those consuming little or none. Cooper (11) however confirmed Maurer's findings, while later both Funk (12) and Cooper and Braddon (13) have brought forward evidence to show that this effect of large quantities of polished rice is due to the large amount of Carbohydrate which it contains.

When no attempts at artificial feeding are made, it is found that the appetite of birds fed on polished rice varies within very wide limits.

Some birds eat greedily until they are no longer able to swallow, their crops, after death, being distended with rice. In the majority of cases, however, the appetite decreases after the first few days and by the time paralytic symptoms appear the daily ration has been voluntarily reduced to 2 or 3 grammes. In rats Drummond (6) has also noted a decrease in the food consumption when the diet is deficient in Accessory factors.

In one instance a pigeon was observed to undergo almost voluntary starvation. Fifteen days after commencing on a diet of polished rice the bird showed signs of paralysis. During the previous twenty-four hours it had only eaten 2 grammes of rice. For the next four days its average consumption did not exceed 0.5 grammes of rice a day. On the day following the appearance of symptoms there was definite evidence of head retraction, but on the third day of the illness there was some improvement and by the fourth day the bird with unruffled feathers was moving freely about the cage. That afternoon however it celebrated its recovery by eating 28 grammes of rice; the following day it was again unwell, though without any definite nervous symptoms. Two days later it died suddenly during a convulsion. Although only an isolated instance it suggests that just as in the case of muscular exertion, the energy expended on digestion and absorption is a factor in hastening the onset of Beriberi symptoms, while the cessation of these activities would appear to allow time for the nutrition of the central nervous to be re-established at the expense of other organs in the body.

6. Bacterial & other infections undoubtedly play some part in shortening the incubation period of Beriberi. In the present instance, the average incubation period for infected fowls was 25.2 days, excluding the bird suffering from Coccidiosis, which as previously stated developed symptoms in  $3\frac{1}{2}$  days.

In pigeons the average period was 20.4 days. Many of the so called fulminating cases of Avian Beriberi in which the symptoms come on with remarkable rapidity and the bird dies in a few hours are found to be infected. McCarrison (2) regards this infection as the cause per se of/



of the fulminating character of the illness. It must however be remembered that it is in these very cases that the most dramatic cures are affected by the administration of Yeast extract, while infected birds suffering from Beriberi do not die with symptoms referable to the infection but with the typical symptoms of Avian Beriberi. These facts and the frequency of *Bacillus coli* infections in experimental birds, post mortem, may possibly indicate that the infections are really of an ante-mortem character. There is however no doubt of the fact that lack of Accessory food factors in the diet by leading to a special type of Chronic inanition renders the body more liable to the attacks of pathogenic bacteria. The question is one of great importance from the point of view of the epidemiologist, for it may serve to explain why an epidemic of for instance Bacillary dysentery should break out in a particular institution at a particular moment. The question of Influenza pandemics is also related to this subject, for it has been suggested not without some show of possibility that the organism or organisms responsible for the disease may have first gained their increased virulence by passing through the bodies of persons debilitated by chronic inanition.

Although one hesitates to apply to man the whole of the results obtained from experiments on animals, it is not improbable that in a study of the various factors influencing the incubation period is to be found an explanation of the somewhat peculiar case incidence which is seen when, as the result of a change in the food supply, an epidemic of Beriberi occurs in a ship or institution. As Wedder (14) remarks "since the average incubation period is about three months, it follows that nothing will happen for about that length of time. When, however, this period has elapsed a few cases of beriberi will begin to appear. These will be those individuals least able to endure the deficiency. As the days go by, more and more individuals will succumb, and when the time has passed at which the great majority can no longer endure the lack of the necessary substance in the food, the cases of beriberi appear in large numbers. Later, when all the individuals of ordinary resistance have succumbed, the "epidemic" gradually decreases in severity, as only here and there a case appears among those individuals who possess much greater resistance to the unfavourable condition than the majority of their fellows". A study of the various factors influencing the incubation period thus throws considerable light on the question of "resistance" to Beriberi.

## 2. THE ONSET AND COURSE OF THE DISEASE.

The main symptoms of the disease will be described as they/

they occur in fowls. Certain differences will be noted in the disease as observed in pigeons.

During the period of incubation there is almost always some diarrhoea. The stools are semi-fluid in character and after the first few days are of a light straw tint, possibly due to a deficiency in bile pigments.

Occasionally the stools are of a bright emerald green colour, due in most cases to an infection with *Bacillus pyocyaneus*.

Fowls dying of inanition do not suffer from diarrhoea and the faeces are of their normal colour.

The actual onset of symptoms is ~~a~~ rule gradual.

The bird's feathers are ruffled and instead of walking about the cage it sits quietly on the ground with its head sunk between its shoulders.

The bird ceases to preen itself and as a result, its feathers quickly become draggled and soiled.

Occasionally the comb becomes cyanosed. When the fowl walks there is a tendency for it to keep the tibio-tarso-metatarsal joint partially flexed. This is due to the commencing paralysis of the extensor muscles of the leg. From the appearance of the first symptom to the time of death is usually from two to three days. In one instance, however, a fowl lived for eight days after the initial symptoms, while in another, death occurred after six hours. Once paralytic symptoms have commenced in the leg muscles, they tend to ascend upwards, involving the muscles of the wing and neck. At the same time the respirations and heart are affected and the temperature gradually falls. As a rule the bird ~~gradually~~ passes into a semi-comatose condition, which terminates in death. Occasionally however death occurs without any warning or in the case of pigeons during a convulsive seizure.

### 3. NERVOUS SYMPTOMS.

(a) Paralysis. Paralytic symptoms are first noted in the legs in every case, the extensor muscles being primarily affected. This is followed by an involvement of the flexor muscles of the toes, as a result of which the bird is unable to grip its perch. As the paralysis of the leg muscles becomes more pronounced the bird is able to walk only with difficulty since it cannot lift up its feet. A few robust birds continue, however, to shuffle about for a time on their flexed tarso-metatarsus. The wing muscles are next affected. This symptom was noted in just over half the fowls and consists in an inability to keep the wings in their usual position close up against the body. In pigeons "wing-drop" was comparatively rare. It was only noted in one instance. This difference in fowls and pigeons may possibly be correlated with the fact that in fowls the muscles involved in flying have undergone partial atrophy from disuse, while in pigeons they are strong and well developed. Weakness in the neck muscles soon appears/



appears. The fowl sits with its head sunk on its breast, while before actual paralysis sets in, it is not uncommon to see the fowl slowly moving its head from side to side, nodding the while as if the head were far too heavy to support. As a rule the anterior muscles of the neck are more affected than the posterior, with the result that head retraction is a very common symptom. It was observed in all the fowls and in all except three of the pigeons. In these three, emprostotonos was noted, the head being bent forward in such a way that the beak touched the ground. At a late stage of the disease, dysphagia or inability to swallow owing to paralysis of the muscles of deglutition was a characteristic symptom seen in nearly every case.

(b) Sensory disturbances. So far as can be judged by experimental means, there is marked impairment of sensation on the legs; touch, pain and temperature sense all being affected. There would also appear to be a slowing of the reaction time and an inability to distinguish whether one or two points are touched at the same moment.

(c) Inco-ordination. The powers of co-ordination are distinctly impaired, for if a tempting grain is held within reach of the bird's beak, it makes several fruitless attempts before it can seize the morsel.

(d) Cerebellar disturbances. Involvement of the Cerebellum is not of common occurrence in fowls, since it only occurred in one case. In pigeons on the other hand, eleven out of fifteen showed symptoms referable to an involvement of the Cerebellum. This involvement is characterised by the occurrence of convulsive attacks of a periodic character. They can be induced by any form of stimulation such as a sudden noise or the flash of a light, much in the same way as the spasms of tetanus. Occasionally the attack begins by fine tremors of the wings, head or eyelids. Minor attacks are characterised by the bird slowly turning round and round in a circle. As a rule the same bird always turns in the same direction in all its attacks. In major attacks on the other hand the bird attempts to turn somersaults backwards. The head is forcibly retracted, the wings extended, while the bird slowly progresses backwards until it comes to rest against the side of the cage. In some cases the bird dies while actually in one of these convulsive seizures, but more commonly the attacks cease some little time before death, the bird passing into a semi-comatose condition from which it can only be raised with difficulty. Possibly these convulsive attacks in the pigeon have some relationship to a derangement of the thyroid-parathyroid mechanism, but on the whole it would seem that/

that they are more probably of Cerebellar origin. In man as in the fowl such attacks are not seen.

(e) Involvement of the Special Senses. Satisfactory deductions are somewhat difficult to make in regard to the activity of the special senses in birds, for even in health there are considerable variations in their visual and auditory powers. On the whole, however, it would seem that a distinct diminution in visual activity does occur in a large number of cases, more particularly during the last stage of the disease; a few cases on the other hand appeared extremely sensitive to light. No impairment of the auditory functions could be detected. In man interference with vision has occasionally been recorded as a symptom of Beriberi. Thus Correa de Bittencourt (15) described changes in the whole of the optic tract in human Beriberi.

2. TEMPERATURE CHANGES. A characteristic feature of Avian Beriberi is the gradual fall in temperature which takes place as the disease progresses. In healthy birds the diurnal range of temperature, taken in the cloaca, is from 105°F to 109°F, the average being 107°F. On a diet lacking in Water Soluble B factor the average daily temperature gradually falls, at first slowly, but in the later stages of the disease more rapidly until it reaches 99°, 98° or even 96°F. If, however, a bird suffering from Beriberi is given a curative dose of Yeast, it will be found that as the paralysis disappears the temperature rises again to 103°F - 105°F. In birds in which, in addition to Beriberi, some septicaemic infection is present the temperature keeps on the average about 103°F or 104°F, not as high as in the healthy bird, but higher than in the Beriberic bird. In man, also, uncomplicated cases of Beriberi always show a normal or sub-normal temperature chart, while the same is true of uncomplicated cases of Pellagra. Two explanations may be suggested for the low temperature met with in Beriberi. In the first place the absence of Water Soluble B factor may exert a direct influence on the cells of the thermic centre, which in the case of rabbits at any rate has been shown by Aronsohn and Sachs (16) to exist in the corpus striatum in close relation to the caudate nucleus. In the second case, in all cases of chronic starvation the metabolic functions are carried on at as low a rate as possible, with the result that the body temperature, which is maintained by the processes of metabolism, falls. In birds fed on a polished rice diet, it would seem that both these explanations are in part correct. The rise in temperature when yeast is given to a Beriberic bird would thus be explained as being due to the direct action of Water Soluble B factor on the thermic centre, while the fact that in this instance the temperature does not rise to its normal height would be explained by/



by the continued effects of reduced metabolism.

### 3. CHANGES IN THE CARDIAC AND RESPIRATORY FUNCTIONS.

A further characteristic of Avian Beriberi is the gradual slowing of the respiratory rate. At sea-level in the tropics, the average number of respirations in the fowl is 54 per minute, but in the later stages of Avian Beriberi this number is reduced to an average of 20-22 per minute. At the same time in the last stage of the disease there is often an appearance as of air-hunger; the bird remains motionless with its beak open, gasping for breath. In fowls but not in pigeons a curious slow bellows-like movement of the abdominal wall has often been noted. When "cured" by a dose of Yeast extract the respiratory rate of birds rapidly returns to the normal nor are there any further symptoms suggestive of air-hunger.

In this connection it is of some interest to note that Remoiné (17) has found that in Beriberic pigeons the respiratory quotient  $\frac{CO_2}{O_2}$  falls to 0.51-0.56, as compared with unity for pigeons fed on entire rice. On administering Yeast, however, the respiratory quotient is at once raised to the normal.

The Cardiac functions are also markedly affected in birds suffering from Beriberi. In the normal fowl the average pulse rate is about 130, though even in health there is frequent variation. In beriberic birds, however, there is as a rule marked acceleration of the heart's action, the rate varying from 180 up to a point at which it becomes quite uncountable. In other cases and these somewhat more rare there is a definite slowing of the rate which falls to 100-110. The slowing, however, is never so marked as the acceleration. Here again the administration of Yeast extract causes a return to the normal. In man the respiratory rate is usually slowed, an average being 18 per minute. The pulse may be sometimes slowed, but much more often is quite rapid, running from 100 up to a quite uncountable rate.

One is greatly tempted to regard all these variations in the cardiac and respiratory functions - the slowing of the respirations, the air-hunger, the acceleration or slowing of the heart - as due, at least in part, to an involvement of the medullary centres of the brain.

Although there is almost undoubtedly a certain change of the respiratory rate due to slowing of metabolism, the lack of Water Soluble B factor would appear to have a definitely paralysing effect on the Cardiac and respiratory centres of the Medulla. In the case of the muscular paralysis of Avian beriberi a rapid cure is produced by the administration of Yeast owing to the direct action of Water Soluble B factor on the motor nerve cells. But no less rapid, though less apparent, is the change in the respiratory and cardiac functions. If the muscular paralysis is removed as the result of direct action on the nerve cells, it would seem only reasonable/

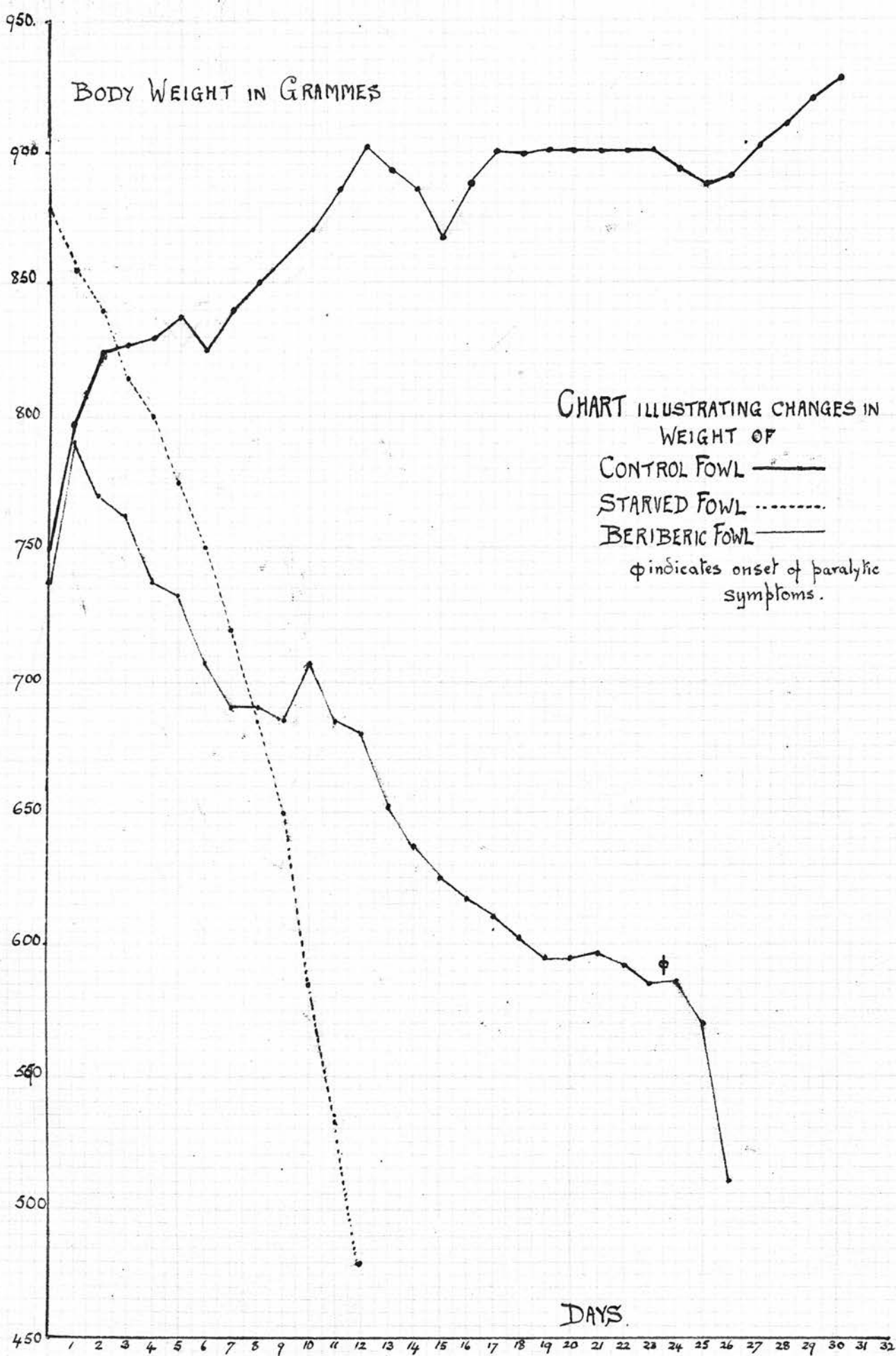
reasonable to suspect that the respiratory and cardiac changes are due to a similar action. If it is conceded that in Avian Beriberi the medullary centres are involved then we have a rational explanation of the paroxysms which serve to bring about a fatal termination in human Beriberi. These paroxysms may occur at any stage of the disease. They are characterised by dyspnoea, cardiac pain and palpitation. The pulse is rapid and feeble, the cervical vessels throb, the face is cyanosed and the body cold. The patient gasps for breath and suffers great praecordial pain until, a few minutes before death, consciousness is lost. The administration of nitroglycerine and amyl nitrite are of little use in such an attack, while bleeding is hardly any better. On the other hand cases which appeared in extremis have been promptly and permanently cured by the administration of extracts containing Water Soluble B factor (Vedder (14) and Andrews (18)).

In the various text books of Medicine it is constantly stated that the cause of death in Human Beriberi is acute heart failure. It would seem, however, that a much more plausible explanation is paralysis of the Cardiac and respiratory centres in the medulla, the more so as quite frequently no degenerative changes can be found in the heart post-mortem. Thus Yamagiwa (19) could detect no changes in the heart muscle in 53.6 per cent of his autopsies, while Miura (20) only found fatty changes in one out of fourteen cases.

4. **OEDAE MA** of the lower extremities was not noticeable during life in any of the fowls or pigeons used in the experiments. The presence of effusions into the serous cavities will be discussed in relation to the histological changes.
5. **CHANGES IN BODY WEIGHT.** When fed on an exclusive diet of polished rice both fowls and pigeons lose weight. Fowls lost 25.6% of their body weight at the commencement of the experiment or 1.2% per diem. Pigeons on the other hand lost 29.0% or 2.5% per diem. As a rule, when first placed on a diet of polished rice birds gain in weight, but by the end of the first week their weight is again equal to that at the beginning of the experiment. In many cases there is a rapid fall in weight shortly before death. In some instances this fall begins before the appearance of nervous phenomena, in others after. Chart 1 shows the changes in weight in a fowl fed on polished rice, in a control fowl and in a fowl dying from inanition. One point of some interest was that in Beriberic fowls the development of secondary sexual characteristics, combs, spurs and tail feathers was inhibited, while towards the end of the experiment the young cocks ceased even to greet the approach of dawn.

(b) Acute/





(b) Acute and Chronic inanition in birds.

In birds fed on an exclusive diet of polished rice symptoms due to chronic inanition are bound to occur. In order therefore to gain some idea as to the part played by inanition in Avian Beriberi, it was necessary to examine firstly birds suffering from Acute inanition and secondly birds suffering from Chronic inanition, but with a full supply of Water Soluble B factor in the diet. In the case of fowls given water only, death took place in an average period of 11.6 days, the shortest period being 3.5, the longest 17 days.

Clinically no signs of involvement of the nervous system were noticeable; progressive asthenia was the main feature. The respirations were slowed but not to the same extent as in Beriberic birds and though a fall in temperature occurred, it was never so marked and in no case fell below 99°F.

The loss of weight at death amounted to 38.7% of the weight at the beginning of the experiment or an average of 6.5% per diem.

As in the case of birds fed on a diet of polished rice there was a failure to continue the development of secondary sexual characteristics.

Out of fifteen birds with which the experiment started three were found post-mortem to be infected with *Bacillus coli*.

As previously described six non-infected pigeons were fed on small quantities of oats and maize and a liberal supply of Water Soluble B factor. Three birds fed on this same diet were infected with *B. coli*.

Two birds survived the full term of the experiment and were killed on the 35th day, while the four others died in an average period of 27 days. Here again there were no paralytic symptoms, but the respirations were slowed and the temperature lowered as in fowls dying from acute inanition. The loss in weight amounted to 26.4% or 1.6% per diem.



## (11) HISTOLOGICAL OBSERVATIONS.

## (a) Avian Beriberi.

The earlier observers of Avian Beriberi, while noting the general atrophy of all the organs, largely confined their attentions to histological observations of the Nervous System, thereby demonstrating the close relationship of Avian and Human Beriberi. Later, however, Funk and Douglas (21) studied the changes in the endocrine glands - observations which have been materially extended by McCarrison. In the present instance the experimental birds were examined immediately after death or were killed when practically moribund. The organs were dissected out, dried on blotting paper and then weighed. Portions of certain organs were fixed in formalin and subsequently sectioned with a freezing microtome to investigate the fatty changes, while the rest of the organs were embedded in paraffin and sectioned at leisure. Before discussing the appearances in the various organs, it will be as well to describe the general post mortem appearances in a typical case of Avian Beriberi:-

Fowl 23. ♂ Weight at beginning of experiment 667 grammes. Weight at death 497 grammes - a loss of 25.4% of the original body weight. The bird showed marked signs of wasting; the feathers were dry and lustreless. The secondary sexual characteristics, combs, spurs and tail feathers were undeveloped. The skin was slightly darker than in healthy birds and there was an entire absence of subcutaneous fat. No petechiae or subcutaneous ecchymoses were noticeable. The muscles were rather dark in colour and were wasted, more especially the pectoral muscles, as the result of which the sternum appeared unduly prominent. There were no actual signs of subcutaneous oedema and no oedema of the muscles. The thymus gland had entirely disappeared. On opening the abdomen the disappearance of the extraperitoneal fat was noticeable as was the atrophic condition of the intestine. The right auricle was dilated, the Auriculo-ventricular band being marked by a thin line of Oedema. The most noticeable feature of the post mortem is thus the extreme degree of Atrophy of all the tissues.

As the result of weighing the organs in beriberic birds and in those dying of inanition it has been possible to estimate the relative degree of atrophy in the two conditions. It will be seen that the degree of atrophy in birds fed on a polished/diet is very similar to that of birds with inanition. In Table 1 is recorded the average weight of the organs of control pigeons fed on an ad libitum diet of mixed grain, of pigeons fed on an ad libitum diet of polished rice and of pigeons fed on a minimal ration of oats and maize with the addition/

/rice

TABLE 1

Showing average Weights of organs of control pigeons, of those fed on polished rice and of pigeons subjected to chronic inanition, but with Water Soluble B factor present in the diet. Weight calculated in terms of original body weight.

Organs.	Control		Rice		Chronic Inanition	
	Average wt. in mgs.	wt. per kilo	Average wt. in mgs.	wt. per kilo	Average wt. in mgs.	wt. per kilo
<i>Male.</i>						
Brain	2267	6894	2070	6855	2116	6872
Adrenals	329	102	52	140	369	120
Thyroid	26	82	22.5	75	23	73
Pituitary	5.9	18.4	5.0	18.7	5.6	18.3
Thymus	748	2318	-	-	-	-
Spleen	347	1072	104	347	9.6	310
Testicles	1489	4610	126	423	201	652
Stomach	5415	26052	5723	19076	5642	17377
Liver	6797	21046	5530	18420	5616	18235
Pancreas	1032	3198	619	2064	611	1988
Kidneys	1751	5422	1562	5207	1635	5311
Heart	3368	10492	2610	8703	2662	8646
<i>Female.</i>						
Brain	1904	7012	2292	6904	2243	7009
Adrenals	236	79	49	147	39	122
Thyroid	25	84.2	24.9	75	21.4	67
Pituitary	5.9	20	6.7	20.5	6.3	19.4
Thymus	361	1209	-	-	-	-
Spleen	313	1049	99	299	98	308
Ovary	288	964	103	311	90	284
Stomach	7197	24070	6113	18414	5520	17252
Liver	6257	20928	6177	18607	5363	16760
Pancreas	1014	3392	704	2123	552	1725
Kidneys	1636	5582	1793	5434	1459	4560
Heart	3072	10276	2888	8700	2759	8624



TABLE 11 - Fowls.

Showing average weights of organs of control fowls, of those fed on polished rice and of those subjected to Acute inanition. Weight calculated in terms of original body weight.

Organs.	Control		Rice		Acute Inanition	
	Average wt. in mgs.	wt. per kilo	Average wt. in mgs.	wt. per kilo	Average wt. in mgs.	wt. per kilo
Male						
Brain	2632	4785	3146	4528	3404	4620
adrenals	113	206	218	315	169	230
Thyroid	43	78	51.5	72	50.8	69
Pituitary	9.5	17	11.6	16.9	12.3	16.8
Thymus	1624	2952	-	-	-	-
Spleen	1499	2725	662	952	795	1078
Testicles	1050	1909	510	734	558	757
Stomach	13170	23945	13512	19396	14118	19156
Liver	19092	34712	21077	30547	22001	29853
Pancreas	1345	2445	1218	1761	1285	1743
Kidneys	4458	8105	5257	7619	5555	7538
Heart	2380	4327	2531	3642	2720	3690
Female.						
Brain	2481	4862	3070	4652		
adrenals	99	187	209	318		
Thyroid	41	78	46	71		
Pituitary	9.5	18	11.8	18		
Thymus	924	1745	-	-		
Spleen	1401	2624	659	998		
Ovary	314	592	246	373		
Stomach	12626	23822	12578	19058		
Liver	18285	34500	20037	30360		
Pancreas	1310	2472	1191	1805		
Kidneys	4264	8046	4992	7564		
Heart	2276	4295	2466	3737		

TABLE 111.

Showing per centage loss of organs of pigeons and fowls fed on polished rice and subjected to inanition; calculated as per centage of weights per kilo of control birds.

Organs.	PIGEONS		FOWLS	
	Rice	Chronic Inanition	Rice	Acute Inanition
Thymus	100	100	100	100
Testicle	82	85	61	60
Spleen	67	71	65	60
Ovary	67	69	37	—
Pancreas	35	37	28	28
Stomach	23	27	19	20
Heart	17	19	15	14
Liver	12	13	12	14
Thyroid	8.5	10.9	8	11
Kidneys	3	2	6	7
Brain	0.56	0.3	5.0	3.0



addition of a plentiful supply of Water Soluble B factor. In Table 11 is shown the average weight of the organs of control fowls, of fowls fed on polished rice and of fowls dying of acute inanition. It will be noticeable that in both species the adrenal undergoes hypertrophy, the pituitary would appear to undergo little or no change, while all the other organs atrophy. The order in which the organs undergo atrophy, with the extent of that atrophy is shown in Table 111. It will be observed that whereas the order in which the organs undergo atrophy is the same both in fowls and pigeons, the actual degree of atrophy of the various organs varies slightly in the two species. In describing the various organs, the systems will be considered in the following order:-

- (1) Nervous System.
- (2) Endocrine Organs
- (3) Genito Urinary System
- (4) Circulatory System
- (5) Alimentary System
- (6) Muscular & Osseous systems.

(1) The Nervous System.

(a) Brain & Cord.

Macroscopically in very many cases, the brains of birds dying of Beriberi exhibit no obvious abnormality. Occasionally, however, there is slight congestion of the meninges and, though more rarely, of the brain substance itself. At other times the brain substance is of a dead white colour, indicative of profound anaemia. In almost every case the brain substance is soft and sometimes almost diffluent. No increase could be found in the Cerebro-spinal fluid nor were the lateral Ventricles every dilated. There were no petechial haemorrhages. The Cord appeared normal in all cases. The weights of the brain were as follows:-

	Males			Fowls			Females		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average Weight in gms.	2.63	3.14	3.40	2.48	2.81	—	—	—	—
Variation in weight	2.62-2.78	2.67-3.49	2.78-3.52	2.41-2.87	2.63-3.12	—	—	—	—
Average Weight per kilo of body weight	4.785	4.528	4.620	4.862	4.267	—	—	—	—
	Pigeons			Females					
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
average Weight in gms.	2.26	2.07	2.11	1.90	2.29	2.24	—	—	—
Variation in weight	2.02-2.31	2.01-2.29	1.89-2.28	1.64-2.12	2.11-2.30	1.86-2.29	—	—	—
Average weight per kilo of body weight	6.894	6.855	6.872	7.012	6.904	7.009	—	—	—

The degree of atrophy both in beriberic and starved birds is comparatively slight, but is rather ~~the~~ more pronounced in the birds fed on an exclusive diet of polished rice.

For the histological examination of the nerve tissues, the following staining methods were employed:-

Haematoxylin and Eosin and Van Gieson for the general arrangement of the tissues, Nissl's and Heidenhain's iron-haematoxylin methods for the nerve cells, Marchi, Heidenhain's iron-haematoxylin and fuchsin and haematoxylin for the nerve fibres.

No macroscopical abnormalities were noted in the brains of control birds or of birds subjected to inanition. In the brains of birds fed on polished rice, the following appearances were noted in

1. The Nerve Cells. The most characteristic change was the almost complete disappearance of the Nissl granules. Instead of being present as angular masses scattered throughout the cell substance they were represented by a fine granular mass collected in one part of the cell, usually close to the cone of origin of the axis cylinder process. In a few cases the cell nuclei appeared pale and showed evidence of vacuolation, but this was comparatively rare. The chromatolytic changes were especially well seen in the cells of the grey matter of the cord, both in the anterior and posterior horns and in the cells of the medulla and pons. Chromatolysis was also noted in pigeons in the cells of the subthalamus region. The degree of chromatolysis is a little more advanced than that seen when a pigeon is fatigued by a long flight. On curing a bird with Beriberic paralysis by means of a dose of yeast, the Nissl granules can be seen to reappear in a few hours time. In birds dying of inanition only slight evidence of chromatolysis could be found in the nerve cells, but it was never so widespread or so marked as in birds fed on polished rice.

2. The Nerve processes. As a rule, in sections of the brain and cord from birds dying of Beriberi one finds a few fibres staining deep black. The distribution of these fibres is entirely capricious; they may occur in any tract. There is no relationship to the blood-vessels or lymphatics. In certain cases, however, no degeneration of the myelin can be demonstrated by Marchi's method. This occurred in three fowls and two pigeons in the present series and was also noted in the fowl suffering from Coccidiosis. All these birds, however, died of typical paralytic symptoms despite the absence of degenerative changes in the fibres.

Marchi's process.

Examination of sections stained with haematoxylin and acid fuchsin showed that in a very few instances, the axis cylinders had become granular and fragmented. In two cases of pigeons which died from chronic inanition/



inanutition with Water Soluble B factor present in the diet, one or two fibres were found staining by Marchi's method.

3. Neuroglia. No abnormality could be found in the neuroglial tissues.
4. Blood Vessels. In a few cases the blood vessels showed evidence of congestion. In the smaller arteries the muscular fibres were fragmented, while the endothelial lining was rather swollen in places, the nuclei being obscured.

(b) The Peripheral Nervous System.

The Sciatic Nerves were examined in all cases. Degeneration of the myelin sheaths could be demonstrated by Marchi's method in all the fowls except three and in all the pigeons except two. By no means all the fibres, however, showed evidence of this definite degeneration, the number of fibres affected varying in different birds and in the two sciatics of the same bird. The average number of fibres affected, however, is always roughly that noted by Vedder and Clark (1) namely ten to fifteen per cent.

In those fibres which did not show actual Wallerian degeneration, changes could nevertheless be detected in the myelin sheath by the use of the iron-haematoxylin method.

In normal nerve fibres stained by Heidenhain's iron-haematoxylin method, the myelin is found to contain numerous small rod-like structures, which appear to be arranged radially round the axis cylinder. When degeneration begins, the first change is a disappearance of the small rods, which now appear as irregularly branched anastomosing globules: later these globules become more distinct until finally they give the typical picture seen in Marchi preparations.

In the three fowls, which though dying of Beriberi, yet showed no degeneration by Marchi the rod like structures in the myelin were, however, seen to be replaced by peculiar irregular globules, when the sections were stained with Heidenhain's iron-haematoxylin method.

In the sciatics of two pigeons dying of chronic inanition, changes could be detected by Marchi's method in a few fibres.

In Beriberic birds the axis cylinder processes of a few fibres showed fragmentation.

The Vagi showed degeneration by Marchi's method in all the fowls fed on polished rice except five and in all the pigeons except three. None of the birds suffering from inanition showed evidence of degeneration of the Vagi.

The Sympathetic Nervous System. Changes indicative of degeneration were found in the Nerve plexuses of the intestine, while the cells of the ganglia of the adrenals were swollen and vacuolated.

Owing to their extreme minuteness, in birds it is somewhat/

What difficult to study the sympathetic nerve fibres satisfactorily.

## 2. The Endocrine Organs.

(a) Adrenals. In fowls the adrenal glands lie in the middle line of the body, separated only from one another by the main vascular trunks. They are roughly pyramidal in shape and are in close relationship to the upper poles of the kidneys, from which they are separated by a layer of connective tissue. They are extremely vascular and receive from the splanchnics numerous small fibres, associated with ganglia which lie in close relationship to the gland tissue. The adrenals are of a yellow ochre colour and of firm consistency; not infrequently the right is a little larger than the left adrenal. In the male they are in close relationship to the postero-internal surface of the testicles, while in the female the ovary overlies the left adrenal and is adherent to it. As a result great care has to be exercised in dissecting out the adrenals. In pigeons the anatomical relationships are practically the same, though the glands are of course smaller.

While in mammals the cortical and medullary elements, which together make up the gland, are comparatively distinct, in birds as in some reptiles such as the Crocodile, there is an interlacement of the cortical and medullary elements. The cortical cells are arranged in cords, always two deep with the blood vessels, while the intervals are filled up with groups of polyhedral medullary cells.

The medullary cells contain numerous small granules, which have a strong affinity for chromic acid and are thus known as "chromaffin" cells, while the cortical cells are laden with lipid substances. The variations in weight of the adrenals in control fowls, beriberic fowls and those dying of acute inanition is shown below, together with the weights of the organs in control pigeons, beriberic pigeons and those dying of chronic inanition, with Water Soluble B factor present.

	Fowls			Pigeons		
	males			females		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mgs.	113	218	169	99	209	-
Variation in weight	97-147	120-380	128-289	92-138	180-401	-
Average weight per kilo of body weight	206	315	230	187	318	-



	Pigeons			Females		
	control	Rice	Inanition	control	Rice	Inanition
Average weight in mgs	32.9	52	36.9	23.6	49	39
Variation in weight	16-52	28-73	14-57	11-38	28-84	12-61
Average weight per kilo of body weight	102	140	120	79	147	122

It will thus be seen that a definite hypertrophy of the adrenals occurs both in fowls and pigeons when fed on a diet of polished rice. The degree of hypertrophy in the two species is approximately the same. In the female the hypertrophy is somewhat greater than in the male.

Hypertrophy though to a less extent also occurs in fowls and pigeons subjected to inanition. The changes noted in the glands of Beriberic fowls and pigeons were very similar. The organs were of a reddish copper hue and were very friable. A considerable degree of congestion was present in some cases. No haemorrhages were found in the adrenals of uninfected birds, but in one case of a fowl infected with *B. coli*, the glands were reduced to a haemorrhagic mass. The medullary cells appeared for the most part normal. Though many of the nuclei seemed quite healthy, a large number exhibited degenerative changes, either staining darkly and uniformly or more commonly showing a loss of chromatin material, the network being solely represented by a thin ring of chromatin placed round the periphery of the nucleus. In some cases the nuclei were enlarged and vesicular in appearance. The chromaffin granules were examined both in sections fixed in potassium bichromate and in portions of tissue fixed in Osmic Acid Vapour as recommended by Cramer (22). They appeared practically normal in numbers, showing no decrease and no increase such as is associated with an increased production of adrenalin.

In three instances dark yellow pigment granules were found scattered throughout the tissues. Even in normal birds the amount of lipoid material present in the cortical cells shows a considerable degree of variation. Only by examining a considerable number of adrenals from normal birds can any true idea of the average amount of lipoid present be obtained. It may, however, be definitely stated that the amount of lipoid present in the adrenals of fowls and pigeons fed on polished rice is definitely in excess of that occurring in control birds. As a result there is a definite enlargement of the cortical cells, a change which can be appreciated by comparing the areas occupied by medullary and cortical cells in sections from Beriberic and control birds.

With/

With Scharlach R the whole of the lipoid stains an orange tint. There does not appear to be any free fat present in the hypertrophied adrenals. If, however, a bird suffering from paralytic symptoms be given a curative dose of yeast and killed some six to ten hours afterwards, a remarkable change will be noted in the cortical cells; the lipoid content has decreased to a remarkable degree, being now less than that normally found in controls. Whereas in healthy birds only a few cells give the Oxidase reaction \* with Vital methylene blue, now it can be obtained in almost every cell.

As a result of this removal of lipoid from the cortex there is a decrease in the weight of the adrenals, a fact already noted by McCarrison though his explanation was of course somewhat different.

In birds dying from inanition the lipoid content is usually increased to a slight extent, but never to the same degree as in birds fed on polished rice. In starved animals congestion was not marked, nor were changes noticeable in the small sympathetic ganglia, though in beriberic birds the ganglion cells show evidence distinctly indicative of degeneration. Nuclear changes were not observed in birds dying from inanition.

\* [The oxidase reaction is obtained as follows:- Small portions of the adrenals of a freshly killed bird are placed in a solution of Vital Methylene blue. The lipoid material takes up the dye. The tissue is then immersed in saline solution and covered with a cover-glass, which is ringed with vaseline. The preparation is ~~then~~ placed in the incubator at 37°C and left there for six hours. After this time all the blue colour has disappeared, but on lifting up the cover-glass and readmitting air the colour returns owing to the fact that the granules use up and take up oxygen from the air. Marinesco was the first to point out that under normal circumstances the adrenal cortex does not give the oxidase reaction, since the lipoid content of the cells stains uniformly and not as granules; it is only when some of the lipoid has escaped that small granules of lipoid are seen and these give the typical oxidase reaction.]

(b) Pituitary. In the pigeon, the pituitary is about the size of a pin's head, while in the fowl it is not quite twice as large.

Owing to the minute size of the gland and the difficulty of extracting it from the sella tursica in which it lies, too much reliance cannot be placed on the weights obtained.

It would appear however, that the pituitary undergoes little or no atrophy either in inanition or in Beriberi. A conclusion which is supported by the very slight changes found in the gland as the result of histological observations/



observations. In the posterior lobe no abnormality could be found, while in the anterior in most cases the only noteworthy change was that in a few of the finely granular cells the nuclei stained poorly; in one or two instances, however, there was a distinct diminution in the number of cells, with the result that the framework appeared to be more prominent than usual.

(c) Thyroid. In birds the thyroid consists of two portions, placed on each side of the neck in close relation to the great vessels just as they emerge from the neck. In the fowl each portion is rather larger than a grain of wheat and is of a delicate pink shade. It is an extremely vascular organ and on removal appears somewhat smaller than when in situ. Histologically even in health the thyroid tissue varies somewhat in appearance; the vesicles are small and irregular in outline, while the intervesicular tissue is large in amount and occasionally forms masses of tissue closely resembling parathyroid. The actual amount of colloid material also shows considerable variation. In birds suffering from Beriberi the degree of atrophy is very slight as is shown by the following figures:-

	<u>Fowls</u> males			Females		
	Control	Rice	Starvation	Control	Rice	Starvation
Average weight in mgs.	43	51.5	50.8	41	46	—
Variation in weight	39-56	42-58	40-56	37-46	37-49	—
Average weight per kilo of body weight	78	72	69	78	71	—
	<u>Pigeons</u> males			Females		
	Control	Rice	Starvation	Control	Rice	Starvation
Average weight in mgs.	26	22.5	23	25	24.9	21.4
Variation in weight	22-31	19-30	18-29	18-31	20-28	17-26
Average weight per kilo of body weight	82	75	73	84.2	75	69

Macroscopically the organ is rather paler than usual. Histologically there is frequently no change to be noted, but occasionally the cells lining the vesicles have a degenerated appearance. In non-infected birds there is no evidence of any inflammatory reaction. In starving birds the amount of colloid is usually markedly decreased.

(d) Parathyroids appeared normal both in beriberic and starved birds except for the disappearance of some of the fat globules contained in the cells. The post-branchial body which is usually anatomically continuous with the parathyroid in pigeons appeared to have undergone some atrophy.

(e) Thymus. The thymus gland in birds is composed of two portions, placed on each side of the neck in close relationship to the oesophagus. The two portions are each composed of a chain of swellings resembling a string of sausages running from the base of the skull to the entrance to the thorax.

The weight of the thymus varies considerably in control birds, but is considerably heavier in the male than in the female.

In fowls and pigeons suffering from Beriberi, no trace of any thymic tissue could be found and the same is true of birds suffering from Acute and chronic inanition, even when Water Soluble B factor is present in the diet.

(f) Spleen. In the fowl, this organ lies immediately to the right of the junction of the glandular and muscular stomach, beneath the peritoneum which forms an investment to it. Normally the spleen is of a reddish brown colour and is surrounded by a capsule composed of fibrous and muscular tissue. In the fowl it is rounded in shape while in the pigeon it is more like a sausage. From the following figures it will be seen that there is a very marked degree of atrophy both in beriberic birds and in those dying of inanition:-

	<u>Fowls</u> <sup>males</sup>			<u>Females</u>		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in gms.	1.499	0.662	0.795	1.401	0.659	-
Variation in weight	0.920-1.634	0.310-0.890	0.450-0.915	0.842-1.620	0.450-0.875	-
Average weight per kilo of body weight	2.725	0.952	1.078	2.624	0.998	-



Pigeons  
Males

Females

Control    Rice    Inanition

Control    Rice    Inanition

Average weight in gms.    0.347    0.104    0.201    0.313    0.099    0.095

Variation in weight    0.097-0.420    0.099-0.220    0.087-0.452    0.125-0.460    0.086-0.15    0.015-0.017

Average weight per kilo of body weight    1.072    0.347    0.652    1.049    0.299    0.308

The atrophy is so great as to amount to nearly two thirds of the original weight of the organ. Macroscopically in birds fed on polished rice, the spleen is usually of a dark red colour and is very friable. Microscopically the Malpighian corpuscles are difficult to make out owing to the disappearance of a large number of the lymphoid corpuscles. As a result the central arteries and fibrous tissue framework appear more prominent than in the healthy spleen. There is also a considerable degree of congestion as a result of which the branched reticulum cells are compressed. Nuclear degenerative changes are noticeable both in the lymphoid cells and the reticular cells, while in many cases a large amount of pigment of a deep yellow colour is scattered in the form of granules throughout the organs. The presence of this pigment was not noted in the spleen of birds dying from inanition nor were nuclear changes seen.

### 3. The Genito-Urinary System.

#### (a) The Genital Organs.

(1) The Testicles in birds are ovoid organs placed ventral to the anterior end of the Kidneys. The left is somewhat larger than the right. They are surrounded by a thin capsule continuous with the supporting framework in the interior of the organ.

From the weights of the testicles and ovaries recorded below it will be seen that the degree of atrophy of the testicles is greater than that of the ovaries.

Atrophy takes place both in birds fed on polished rice and in those suffering from inanition, even when Water Soluble B factor is present in the diet.

Males (Testicles)    Fowls    Female (Ovaries)

Control    Rice    Inanition    Control    Rice    Inanition

Average weight in mgs.    1050    510    558    314    246    -

Variation in weight    870-1133    214-645    210-670    215-462    210-275    -

Average weight per kilo of body weight    1909    734    757    592    373    -

## Pigeons

Males (Testicles)      Females (Ovaries)

Control   Rice   Inanition   Control   Rice   Inanition

average weight in mgs.	1489	126	201	288	103	90
variation in weight	1020-1675	110-179	116-274	250-306	97-106	84-107
average weight per Kilogram body weight	4610	423	652	964	311	284

In healthy adult birds, the various layers of cells lining the seminiferous tubules may be distinguished with ease. Next the membrana propria are the spermatogonia or parent germ cells, with syncytial cells between them. These syncytial cells are larger than the spermatogonia and are rich in lipoid material; internal again to the spermatogonia are other cells showing active mitosis - the spermatocytes - which give rise to the small spermatids; these can be seen in process of differentiation into spermatozoa. In the interstitial tissue are seen groups of polygonal cells - the cells of Leydig - which also show the presence of lipoid granules. In the testicles of birds dying from Beriberi the centre of the tubules was found to be filled with cellular debris; the tubules were shrunken and as a result the interstitial tissue appeared to be increased in amount. A few spermatozoa could still be distinguished, but their heads did not take on any basic stain. No clear differentiation could be made between spermatogonia, spermatocytes or syncytial cells, for many of these had lost their lipoid globules. The tubules were lined by a single layer of undifferentiated cells. The interstitial cells of Leydig still contained a certain amount of lipoid material, but in many cases the nuclei were swollen and vesicular.

(2) The Ovary. This organ is placed on the left of the middle line, partly ventral and partly cranial to the left kidney. The left adrenal is closely applied to a portion of the posterior surface. Macroscopically the organ is small, the vesicles all being minute and of about the same size. Microscopical examination in pigeons fed on polished rice showed the membrana granulosa to be composed of a single layer of epithelial cells, while the nuclei of some of the interstitial cells showed degenerative changes. Very similar appearances were noted in birds dying of inanition.

(b) The Kidneys. The kidneys in birds are each composed of three or four lobes. They are of a deep purple colour and show considerable variation even in healthy/ in weight



healthy birds:-

	Males.			Fowls			Females.		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mgs.	4458	5257	5555	4266	4992	-			
Variation in weight	3820-5365	4526-5980	4730-6120	3545-4660	3820-5463	-			
Average weight per kilo of body weight	5105	7619	7538	8046	7564	-			
	Males.			Pigeons			Females.		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mgs.	1751	1562	1635	1636	1793	1459			
Variation in weight	1524-1779	1516-1604	1522-1695	1470-1690	1537-1809	1420-1485			
Average weight per kilo of body weight	5422	5207	5311	5582	5434	4560			

It will thus be seen that compared with some other organs there is relatively little atrophy. Nevertheless there are quite definite microscopical changes present in the kidneys in birds fed on a diet of polished rice. Congestion as a rule was not very marked, though in a few cases the intertubular capillaries were distended with blood. The glomeruli for the most part were normal and there was no increase in connective tissue. No signs of an inflammatory reaction were noted. Degenerative changes were most marked in the cells of the convoluted tubules. The cells were swollen and granular and projected into the lumen of the tubules, which were thus partially obscured. In some cases the cell protoplasm was actually breaking down. Actual fatty degeneration was not encountered in any of the cases examined. The nuclei in a few instances showed over-staining, but much more frequently evidence of chromatolysis. The cells of the straight tubules were much less affected, though even here, some of the nuclei were undergoing degeneration. In three cases a few yellowish pigment granules were found scattered throughout the tissues. In inanition the changes were very similar to the above, except that nuclear degeneration was much less in/

in evidence.

#### 4. The Circulatory System.

(a) The Heart. The heart varies very considerably in weight both in healthy and diseased birds. The changes in weight are as follows:-

	<u>Fowls</u>			<u>Females</u>		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mg.	2350	2531	2720	2276	2466	-

Variation in weight 2010-2854 2125-2645 2345-2902 2140-2470 2200-2845 -

	<u>Fowls</u>			<u>Females</u>		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight per kilo of body weight	4327	3641	3690	4295	3937	-

	<u>Pigeons</u>			<u>Females</u>		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mg.	3368	2610	2662	3072	2688	2759

Variation in weight 2408-3560 2302-2900 2415-2840 2462-2950 2210-2946 2324-2950

	<u>Pigeons</u>			<u>Females</u>		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight per kilo of body weight	10492	8703	8646	10276	8700	8624

Macroscopically there are certain highly characteristic changes met with in the hearts of birds dying from Beriberi. The right heart is frequently dilated - most commonly the auricle only is affected, but occasionally both auricle and ventricle show dilatation. In two cases in fowls in which both auricle and ventricle were dilated, 10 c.cs and 12 c.cs of fluid were obtained from the pericardial cavity.

The heart muscle was very pale in colour and lacked firmness - the walls of both auricles and ventricles were thinned and frequently a line of oedema could be seen at the auriculo-ventricular band. Not infrequently the right heart was filled with an antemortem clot, which in some cases extended into the great veins. Microscopically the myocardial cells exhibited cloudy swelling/



swelling and in some few cases commencing fragmentation, while changes indicative of nuclear degeneration were of frequent occurrence.

Oedema of the whole heart muscle was met with in one fowl, while in four others it was restricted to the area of the auriculo-ventricular band.

In inanition very similar changes were met with both macroscopically and microscopically - dilatation of the whole of the right heart was, however, of rare occurrence and was only met with in one fowl dying of acute inanition and one pigeon dying of chronic inanition.

In each case there was fluid in the pericardial cavity.

Oedema at the auriculo-ventricular junction was seen in three fowls and three pigeons dying from inanition.

Hydropericardium. The amount of fluid present in the pericardial cavity varied from one drop to 12 c.cs in fowls and from one drop to 4 c.cs in pigeons.

Hydropericardium was present in

4	out of	15	cases	or	26%	of	fowls	with	Beriberi
3	"	"	15	"	"	20%	"	pigeons	"
2	"	"	12	"	"	16%	"	fowls	with
1	"	"	6	"	"	16%	"	pigeons	"

It will thus be seen that an increase of fluid in the pericardial cavity occurred in pigeons even when Water Soluble B factor was present in the diet.

(b) The Blood Vessels. There were no signs of arterio-sclerosis in the aorta or larger arteries. On examining the smaller arteries microscopically there was noted swelling of the intima, while in the media, degenerative changes were found in the muscular coat.

## 5. The Alimentary System.

(a) The Aesophagus is markedly thinned. The epithelial cells have in some places almost completely disappeared while the muscularis mucosae is atrophied; the cells of the small aesophageal glands are swollen and granular and in many cases the nuclei stain poorly. The circular and longitudinal muscle fibres also show atrophic changes.

(b) The Crop exhibits practically similar changes to those encountered in the aesophagus.

(c) The Stomach of the fowl and pigeon consists of two portions:-

(1) The Glandular Stomach is lined internally by columnar epithelium between which are the openings of numerous simple and relatively short tubular glands. A distinct muscularis mucosae is present together with a certain amount of lymphoid tissue. Deep to the muscularis mucosae is a peculiar layer of glands.  
Each/

Each gland is of a simple tubular character and is lined by low columnar cells. The glands are arranged in such a way that their lumina all drain into a single cleft, which after traversing a narrow duct opens on to the surface of the mucous membrane.

In Beriberic birds the glandular stomach appeared to be only slightly thinned: congestion was often marked and in three instances small petechial haemorrhages could be clearly distinguished. The columnar cells lining the mucous membrane were swollen or even entirely atrophied. The cells of the short tubular glands were swollen and the muscularis mucosae thinned. No patches of lymphoid tissue could be seen: apparently they had disappeared coincidentally with the atrophy of the other lymphoid structures in the body. The tubular glands opening into the common cavity were also atrophied: the low tubular epithelium was either swollen or breaking down, and active secretion appeared to have ceased.

In birds dying from inanition the appearances were very similar. In fowls especially small portions of grit were frequently found in the glandular stomach, and in one fowl one of these small stones had become firmly embedded in the mucosa thus leading to the formation of a definite Ulcer.

(2) The Muscular Stomach or gizzard is in shape somewhat like a bi-convex lens. Externally it is covered by a thin tendinous layer, thick at the centre of the organ and thin at the margins. Internal to this is a layer of muscle, deficient at the centre however, but thickest at the upper and lower extremities of the organ. Internally the glandular stomach is lined by a thick horny substance, raised into ridges: beneath this is a layer of long tubular glands which secrete the horny substance. These glands are separated from the muscular fibres by a layer of connective tissue. Both in Beriberic birds and in those dying from inanition, there is a considerable degree of atrophy in the muscular stomach. The weights of the organ are shown below:-

	Males			Fowls			Females		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in gms.	13.17	13.51	14.12	12.62	12.58	-			
Variation in weight	12.12-14.26	12.46-14.41	12.10-14.65	12.01-14.20	12.13-14.09	-			
Average weight per kilo of body weight	23.94	19.39	19.16	23.82	19.01	-			



Males.			Pigeons			Females.		
Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in gms.	8.42	5.72	5.64	7.19	6.11	5.52		
Variation in weight	5.34-8.64	5.20-7.90	5.28-8.16	4.92-8.37	5.19-8.26	5.10-8.69		
Average weight per kilo body weight	26.052	19.076	17.377	24.070	18.414	17.252		

Not infrequently it is comparatively easy to detach the Mucosa from the subjacent muscular tissue. In the birds infected with *Bacillus pyocyaneus*, the horny layer was stained a bright green. Microscopically this horny layer is seen to be diminished in thickness while the subjacent glands are atrophied. The loss in weight would seem, however, to be chiefly due to the atrophy of the muscular tissue.

(d) The Intestines. The intestinal tube varies very slightly in width throughout its entire length; nevertheless a small and a large intestine are usually distinguished. In the small intestine the duodenum is quite distinct, but there is practically no demarcation between jejunum and ileum. The large intestine is sometimes divided into a colon and a rectum. At the junction of the small and large intestine are two tubes with blind ends, the caeca. The intestine is composed of the same layers as in the mammal. In the duodenum and parts of the caeca the mucous membrane is thrown into definite folds covered by columnar and goblet cells. In the other parts are villi. Lymph nodules are present throughout the mucous membrane, especially in the caeca. In Beriberic birds the atrophy of the intestinal wall is so great as to be quite noticeable with the naked eye. No fat is visible macroscopically. The duodenum is congested and frequently ecchymosed. In Egypt intestinal worms were often found in large numbers; both flat worms, which were always dead, and round worms which were active. They were found both in controls and in birds dying from Beriberi and inanition. The round worms especially seemed to flourish in birds fed on polished rice and in one case the intestine was almost blocked by a struggling mass of worms. In this country intestinal worms are not of common occurrence either in control or experimental birds.

In birds dying from inanition congestion of the duodenum was not especially marked. Microscopically the columnar mucous membrane was found/

found to be atrophied; the blood vessels were congested and small haemorrhages were frequently seen. In Beriberic birds the nerve plexus showed evidence of degeneration. The lymphoid tissue had largely disappeared from the intestine and only a very small quantity was to be seen in the caeca. The muscular fibres were atrophied. Inflammatory areas were not encountered in non-infected birds.

(c) The Liver. In healthy pigeons and fowls, the liver is found to be of a light chocolate colour with darker mottlings. In Beriberic birds the usual tint is a dark red or purple. The liver substance is soft and fragile.

In Beriberic birds and those suffering from chronic inanition, the gall bladder is as a rule empty, but in fowls dying from acute inanition it is usually full. The changes in weight are seen below -

	Males.			Fowls		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in gms.	19.09	21.07	22.00	18.28	20.04	-
Variation in weight	18.01-22.45	18.26-22.60	18.51-22.45	18.05-21.96	18.12-22.86	-
Average weight per kilo of body weight	34.71	30.55	29.85	34.50	30.36	-
	Males.			Pigeons		
	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in gms.	6.79	5.53	5.61	6.26	6.18	5.36
Variation in weight	5.34-7.02	5.25-6.98	5.46-6.75	5.34-6.83	5.20-6.46	5.31-5.60
Average weight per kilo of body weight	21.05	18.42	18.24	20.93	18.61	16.76

In adult birds there is no differentiation into lobules as occurs in mammals; the polyhedral cells are collected into relatively large masses, closely packed together, separated only by blood vessels of some size. In the chicken, however, it is possible to distinguish traces of a lobular arrangement and to make out the presence of sinusoids.

In Beriberic birds there is always marked congestion, as a result of which the liver cells are compressed.

The/



The actual condition of the cells varies somewhat; in many cases they only exhibit cloudy swelling, but in others marked fatty degeneration is seen and there is evidence that the cell protoplasm is breaking down. Many of the nuclei are altered; a few stain very deeply, while others are pale, swollen and vesicular. Glycogenic degeneration was not found in any case. Occasionally small yellowish granules of pigment were seen scattered throughout the tissues.

(d) The Pancreas. In healthy birds the pancreas is of a delicate salmon pink colour, but in birds suffering from inanition and from Beriberi it is usually dead white. Not infrequently necrotic areas are seen. The weights of the organ are as follows:-

	Males.			Fowls			Females.		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mgs.	1345	1218	1285	1310	1191	-			
Variation in weight	1216-1352	1189-1244	1192-1298	1242-1349	1176-1214				
Average weight per kilo of body weight	2443	1761	1743	2472	1805				
	Males.			Pigeons			Females.		
	Control	Rice	Inanition	Control	Rice	Inanition	Control	Rice	Inanition
Average weight in mgs.	1032	619	611	1014	704	552			
Variation in weight	876-1142	598-624	586-629	845-1069	602-734	546-582			
Average weight per kilo of body weight	3198	2064	1988	3392	2123	1725			

It will be seen that there is a considerable degree of atrophy both in beriberic birds and in those dying of inanition. Microscopically in Beriberic birds the cells lining the alveoli are swollen and cloudy. Many of the nuclei are pale and vesicular. In fowls dying of acute inanition there appeared to be a slight increase in the islets of Langerhans - but in beriberic birds and in pigeons dying of chronic inanition, this increase was not noticeable. Degenerative changes were not marked in the cells of the/

the islets of Langerhans.

#### 6. The Muscular and Osseous Systems.

In birds fed on polished rice the muscles were soft and rather dark in colour. Microscopically there was loss of cross striation and atrophy, while the nuclei were poorly stained.

The bones and joints were examined in a few instances. The bones had obviously taken part in the general atrophy and were lighter than normal; there were no signs of sub-periosteal or other haemorrhages such as are commonly seen in scurvy.



## CONCLUSIONS.

1. The histological changes produced by a diet of polished rice are similar in fowls and pigeons.
2. The histological changes produced in fowls and pigeons by inanition are similar, whether the inanition is due to absolute starvation or to an inadequate diet containing Water Soluble B factor.
3. The histological changes produced in fowls and pigeons by a diet of polished rice are in the main similar to those produced by inanition, but may be differentiated by -
  - (a) the evidence of nuclear degeneration in all organs.
  - (b) the great hypertrophy of the adrenal cortex.
  - (c) the marked chromatolysis in the cells of the Central Nervous system.

## (b) Human beriberi.

For the sake of comparison the post-mortem findings in a typical case of Human Beriberi are appended:-  
 R.L. male aged 30, Hindu - a stoker from S.S. "Inverness" died 2.7.19 at Port Said after an illness of twenty days. Post mortem - four hours after death.

Oedema was noticeable over the legs, thighs, abdomen and back and on the cheeks.

On cutting into the subcutaneous tissues there was marked exudation of serous fluid; the muscles were pale and oedematous; the subcutaneous fat was decreased in amount and rather yellow in that. The left pleural cavity contained 40 c.cs of a clear yellow amber fluid, which was sterile on culture. The right pleural cavity contained 5 c.cs, the pericardial cavity 10 c.cs and the abdominal cavity 10 c.cs of a similar fluid.

Heart. The right side of the heart was dilated, the right ventricle hypertrophied; ante-mortem clots were present in the right auricle; the heart muscle was soft and oedematous, pale and fatty in appearance; the valves were patent. Microscopically the muscle fibres showed fatty degeneration and fragmentation. The nuclei were poorly stained; the small vessels were congested.

Lungs. The bases of both lungs were congested and oedematous.

Liver. This organ presented a typical nutmeg appearance; microscopically there was considerable congestion with necrosis and fatty degeneration in the parenchyma, more especially in the centre of the lobules.

Stomach. The mucous membrane was congested and at the pyloric end were a number of small capillary haemorrhages. The mucosa and muscular layers had undergone considerable atrophy.

Intestines. The duodenum showed a catarrhal inflammatory condition with numerous petechial haemorrhages; the remainder of the intestine appeared normal except for the very considerable degree of atrophy.

The Pancreas exhibited congestion and atrophy with small areas of necrosis more particularly at the head.

Spleen. This organ was of normal size and consistency. Microscopically many of the lymphoid corpuscles were found to have disappeared. The blood vessels were congested.

Kidneys. The Kidneys were small and cyanotic; there was congestion of the intertubular capillaries with swelling and breaking down of the cells of the parenchyma, more especially in the convoluted tubules.

Pituitary. This organ was normal in size and showed evidence only of slight degeneration in the cells of the anterior part.

Thyroid. This gland was rather small and pale and showed signs of commencing atrophy. Many of the nuclei were swollen and vesicular.

Adrenals/



**Adrenals.** These organs appeared larger than normal; they weighed 4.25 grs. and 4.02 grs. respectively. On examining sections, the cells of the zona fasciculata and zona reticularis were loaded with lipoid, while many of the medullary cells also contained lipoid material.

**Nervous System.** The meninges were congested and oedematous. There was an increase in the Cerebro-spinal fluid and the lateral ventricles were slightly dilated. The brain substance itself was pale and rather soft.

Microscopically there was marked chromatolysis in the nerve cells with degenerative changes in many of the nuclei, which were vesicular, eccentric or even undergoing extrusion. A few fibres were found exhibiting Wallerian degeneration. Wallerian degeneration was also seen in the sciatic, musculo-spiral and vagus nerves and in the cervical sympathetic.

### (c) Beriberi in man and animals compared.

Before discussing the histological findings and the deductions to be drawn therefrom, it is obviously of importance to consider whether such deductions can be applied to man, in other words whether Beriberi in man and the disease produced in birds by feeding ~~them~~ on polished rice are one and the same.

It will be necessary therefore to compare the etiological, clinical and histological factors in man and experimental animals.

When men are fed on polished rice under experimental conditions symptoms of paralysis begin to appear, as shown by Fraser and Stanton (23), after ninety days. Paralytic symptoms may also be produced in many other animals by feeding on polished rice or on some other dietary from which Water Soluble B factor has been totally excluded. Thus Schaumann (24) produced these symptoms in monkeys, dogs, rats, guinea pigs and a goat, Braddon (25) produced it in a pig, Shiga and Kusama (26) Tsuzuki (27) Harden and ~~de~~ Silva (28) and McCarrison (29) produced it in monkeys. Andrews (30) produced it in puppies and Drummond (31) in rats.

It will thus be seen that by excluding a certain substance from the diet symptoms referable to the nervous system can be produced at will in a very large number of animals after a certain period has elapsed, this period varying in the different experimental animals employed.

By adding Water Soluble B factor to the diet paralytic symptoms may be prevented. From the etiological standpoint, therefore, Beriberi in man and the disease produced in birds by a diet of polished rice are closely alike.

Turning now to the clinical symptoms it is found that in man the chief symptom is muscular paralysis accompanied by interference with the Cardiac and respiratory/

respiratory functions. In birds the same symptoms are seen, but here the interference with the cardiac and respiratory functions is progressive and leads to a fatal termination in from two to four days, while in man a sudden interference with the cardiac and respiratory functions may lead to death at any time after the onset of paralytic symptoms.

Subcutaneous oedema occurs in the "Wet" form of Beriberi in man, but a number of cases of Human Beriberi do not show subcutaneous oedema at any time. Beriberi in small children, infantile Beriberi, is always of the wet type. In monkeys, subcutaneous oedema is not very common, but has been recorded in two cases by Shiga & Kusama (26). Andrew's puppies showed oedema in every case. In birds subcutaneous oedema is sometimes seen and has been noted by Shiga & Kusama (26), and by McCarrison (2) who believes that it is more common in young than adult birds. Hydropericardium is found occasionally both in birds and man. In pigeons but not as a rule in fowls, attacks of a cerebellar character are common, such attacks do not occur in man. In regard to the treatment of Beriberi, it has already been stated that the administration of a substance such as Yeast containing Water Soluble B factor cures the muscular paralysis with rapidity and markedly improves the cardiac and respiratory functions. In the case of human Beriberi Water Soluble B factor produces marked improvement in the muscular paralysis (Vedder (14) Willcox (32) Findlay (33) ), while the symptoms referable to the cardiac and respiratory systems are rapidly improved (Vedder (14) & Andrews (30)). Taking into consideration the dissimilarity of the species it will be seen that the clinical symptoms are very much alike in man, fowls and pigeons. The similarity of the histological symptoms is also very striking.

In man the nerve cells of the central nervous system exhibit degeneration as evidenced by chromatolysis, enlargement and vacuolation or in some cases extrusion of the nucleus. In birds the only evidence of degeneration is chromatolysis.

Possibly this difference in the nerve cells may be correlated in the first place with the length of time the paralytic symptoms have endured, - in birds from 2 - 4 days, in man some weeks or possibly months, and secondly with the difference in reaction to exhibition of Water Soluble B factor in curative doses. In man the cardiac and respiratory functions are dramatically improved, for the cells of the cardiac and respiratory centres are only attacked as a terminal symptom of the disease, while the cells of the motor centres have been attacked for a considerable period. In birds the degenerative changes have not proceeded very far in any of the nerve cells, with the result that the curative effect is seen in all the nerve cells.

In man the nerve fibres exhibit typical Wallerian degeneration while the same is true of birds. In the adrenals of birds there is found an hypertrophy of the cortical substance which is packed with lipoid, while in/



in man not only the cortical but some of the medullary cells are found to contain lipoid. McCarrison (29) has recorded hypertrophy of the adrenals in monkeys fed on polished rice.

The thyroid shows a very slight degree of atrophy both in man and birds while the pituitary exhibits scarcely any change.

In birds the Thymus has completely disappeared while in Infantile Beriberi it persists and may even show an increase in cellular elements.

In the Spleen, there is congestion with disappearance of many of the lymphoid elements both in man and birds, while in the latter atrophy is a prominent feature.

The Kidney exhibits identical changes both in birds and man, the cells of the convoluted tubules being markedly degenerate.

The heart in man is as a rule hypertrophied, more especially as regards the right ventricle; this hypertrophy is associated with dilatation of the right heart, while the muscle fibres show fatty degeneration.

In the bird the right auricle is usually dilated and occasionally also the right ventricle, but hypertrophy is never seen and fatty degeneration is rare. This is perhaps the most striking difference between the histological findings in human and avian beriberi.

The changes in the liver - atrophy, fatty degeneration and necrosis - are identical both in man and birds, while in both also there is atrophy of the pancreas.

In the stomach in both cases there is atrophy and congestion, while the marked congestion of the duodenum is seen both in birds and man. With certain small differences there are thus many points of similarity between Avian and Human Beriberi. In etiology, in clinical symptoms and in histological appearances, they are closely alike. As a result it is now the almost universal opinion that human Beriberi and the disease produced in birds by a polished rice dietary are one and the same condition.

### (3) PATHOGENESIS

#### - A critical Discussion.

The more recent work on Nutrition, in addition to proving the existence of Accessory food factors, has shed considerable light on the constitution of an adequate diet. Feeding experiments seem to have proved that all known tissue constituents can be synthesized from the amino-acids of protein with the other basal foodstuffs. For provided the accessory food factors are present animals can be adequately nourished on a diet containing only pure protein, fats, carbohydrates and salts. Substances such as nucleic acid, the phosphatides, cholesterin, creatin must then be synthesized by the body tissues.

A new conception of the value of protein as a food has also been obtained. Although assimilated protein may possess energy value, only certain of its amino-acids present nitrogen in a form which the tissues can use to build up their substance and replace wastage. Whereas it was found that the minimal amount of animal protein, which enables an experimental subject to maintain nitrogenous equilibrium is 30 grammes, vegetable proteins must be given in amounts varying with their source to such an extent that equilibrium cannot be maintained on a lesser daily intake than, e.g. 34 grammes of Rice protein.

As a result a scale of protein equivalents as compared with that of animal protein taken as unity has been drawn up and the term "Biological Value of Protein" has been introduced. The importance of this conception has been demonstrated by Wilson (34) in connection with the etiology of Pellagra.

Much work still requires to be carried out on the relation of Amino-Acids to deficiency diseases. At the present time there is perhaps a tendency to attach too much importance to the lack of Accessory food factors in the diet and too little to the possible effects of an insufficient supply of Amino acids.

Now although fowls and pigeons develop Avian Beriberi when fed on polished rice with remarkable rapidity and regularity, nevertheless there are certain disadvantages in this method in that rice itself is unsatisfactory as a staple article of diet. Even when accessory food factors are added pigeons and fowls fed solely on polished rice lost weight, while to the excessive amount of rice in their diet McKay (35) attributes the poor physique of many of the native Indian races. Although the composition of rice varies somewhat in accordance with its source, the/



following may be taken as an average composition:-

Water .....	11.05%
Protein .....	6.62%
Carbohydrate....	80.79%
Fat .....	0.50%
Ash .....	1.04%

It will be seen that polished rice is deficient in protein, fat and inorganic salts, but contains an excess of Carbohydrate. It also lacks Fat Soluble A factor and probably the Anti-Scorbutic factor. In addition, Atton and Hoeson (36) have shown that in the case of man at any rate the co-efficient of protein absorption of rice decreases steadily when the amount of rice in the diet increases. This fact taken in conjunction with the small amount of rice consumed by pigeons after some days on a diet of polished rice, indicates how great must be the degree of starvation in any bird that has died from Beriberi.

If then we are to arrive at a true conclusion as to the rôle of Water Soluble B factor in the vital economy, we must first eliminate as far as possible the pathological changes due to an insufficient supply of salts, proteins and fats, an excessive supply of Carbohydrate and an entire absence of Fat Soluble A and the Anti-Scurvy factor.

In 1913 Gibson (37) found that a partial compensation of the deficient mineral salt content of a diet of polished rice did not prevent, though it appeared to delay slightly, the onset of paralytic symptoms in fowls. Chamberlain, Bloombergh and Kilbourne on the other hand found that the salts of phosphorus or potassium added to the diet had no effect either in preventing or delaying the onset of Beriberi. The deficiency in salts does not, therefore, seem to play a very prominent part in the pathogenesis of Beriberi. The lack of protein undoubtedly contributes largely to the general state of inanition from which Beriberic birds suffer. This lack is due to two factors - the small protein intake and the insufficient variety of the Amino acids present in Rice. This lack of variety in the Amino Acids may, however, be discounted by comparing the histological findings in Beriberic birds ~~on the one hand~~ with those fed on Water only and on a mixture of maize and oats, the Amino acids of which are of course different from those in rice.

The addition of protein to the diet does not defer the onset of Beriberi in birds, in fact an excess of protein has rather the reverse effect, for as Funk (12) has shown, if pigeons are fed on polished rice with the addition of an excess of the various food stuffs, the incubation period is considerably shortened by the addition of excess of Starch or Sugar, slightly shortened by an excess of protein and unaffected by an excess of/

of fat. The affect of an excess of Carbohydrate in decreasing the body content of Accessory food factors has been noted not only in Beriberi but by Mellanby (38) in connection with the Anti-rachitic factor.

The question of the fat metabolism is also of some interest. As was noted in describing the post mortem findings in Avian Beriberi there is a total disappearance of all naked eye fat. This is of importance when the main facts of hibernation in mammals are reviewed.

Hibernation and its analogue, aestivation, are brought about primarily not by changes in the external temperature but by changes in the food supply. Before hibernating, animals store up large quantities of fat. Thus the Raccoon dog (*Nycterotes procyonoides*) hibernates during the winter only if the food supply during the autumn has been sufficient to allow it to store up a sufficient supply of fat.

During hibernation, metabolism is reduced to a very low level and fat is absorbed, but hibernating animals do not develop Beriberi. Birds, it should be noted, do not hibernate; one cannot however help wondering whether the torpid condition, the falling temperature and the lowered respiratory rate are not, at least in part, an attempt on the part of the tissues to approximate to the hibernating state.

In regard to the action of the Anti-Scurvy Vitamine in birds little is known. It will be remembered that certain animals, e.g. Rats, do not seem to feel the lack of the Anti-Scurvy factor. During the present experiments small doses of orange juice were given to two pigeons fed exclusively on polished rice, but without modifying the clinical symptoms or histological findings.

The part played by Fat Soluble A factor in the pathogenesis of Avian Beriberi is also unknown. Certainly none of the experimental birds developed the curious eye condition, known as Xerophthalmia, which is regarded by many authorities as pathognomonic of a deficiency of Fat Soluble A, and which has also been noted in children in Denmark by Bloch (39) and others; nor did the experimental birds show any particular liability to respiratory diseases. The absence of these pathogenic changes in birds and their very constant occurrence in mammals does not, of course, prove that Fat Soluble A is unnecessary to birds, but it does seem to show that in birds fed on polished rice for a period up to 40 or 50 days, the lack of Fat Soluble A factor does not cause any obvious pathological lesion.

Since then inanition plays so large a part in the etiology of Avian Beriberi, it is not surprising that many of the tissue changes are essentially similar to those in inanition. By studying the changes due to complete inanition and to inanition when the birds are fed on oats, maize and Water Soluble B factor it is possible to eliminate to a certain degree the changes/



changes in Beriberi brought about by lack of salts, amino acids and fats and by excess of Carbohydrate. In order, therefore, to arrive at a conclusion as to the part played by Water Soluble B factor in normal metabolism it will be necessary to discuss critically the functions of the various organs in health, their impairment in Beriberi and their changes in inanition.

1. The Nervous System. Since from the clinical point of view the main symptoms of Beriberi are referable to the nervous system, it is perhaps not surprising that the earlier observers confined their histological observations to this system. The discovery of Wallerian degeneration in the peripheral nerves by Scheu~~ke~~ (40) and Baelz (41) led to the general conclusion that Beriberi is a peripheral polyneuritis. That this conclusion is however untenable is shown by the following facts:-

- (1) Degeneration can be demonstrated in the nerve sheaths some days before the onset of paralysis (Vedder and Clark (1)).
- (2) The administration of Water Soluble B factor cures the symptoms of paralysis in a few hours in birds, but does not repair the degeneration in the myelin sheath.
- (3) Symptoms of Beriberic paralysis can occur when no degenerative changes can be found by Marchi's method in the myelin sheaths, as shown by McCarrison (2) and confirmed in the present experiments.

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Since then the essential lesion is not/be found in the peripheral nerves, attention is naturally directed to the central nervous system. Now the only changes constantly present in the central nervous system of Human and Avian Beriberi are confined to the cell bodies. In man chromatolysis and nuclear degeneration are widespread, while in birds the process is not so far advanced and chromatolysis only is the main feature. As was previously pointed out the administration of Yeast to a bird with Beriberic paralysis is promptly followed by a disappearance of the paralysis, but at the same time there is a re-appearance of the Nissl granules in the nerve cells. There is, therefore, presumptive evidence that the paralysis is in some way connected with the disappearance of the Nissl granules. Now it is a well known fact that if an animal is fatigued by muscular work there is at first an increase of Nissl granules, but later an almost entire disappearance, until after a period of rest the Nissl granules reappear. These granules are regarded by most authorities (Schafer (42) Halliburton (43)) as composed of nucleoprotein. Mott (44) however/

however, has found that when living nerve cells are examined with the ultra-microscope no Nissl granules are seen - the cell is filled with small colloidal globules about  $1\frac{1}{2}$  in diameter, which may possibly give rise to the Nissl granules when the cell is fixed and stained. Without however entering into the question of the exact precursors of Nissl granules in the living cell, it may be taken for granted that, since the metabolism of the neurone is presided over by the nucleus, the appearance or non-appearance of the Nissl granules in a fixed and stained cell is an indication of the activity of the nucleus of that cell during life. Marked chromatolysis is then a sign of nuclear inefficiency - nuclear starvation, and as a result the paralysis of Beriberi, is in some way associated with Nuclear Starvation.

## 2. The Endocrine Organs.

(a) The Adrenals. One of the most interesting changes in Avian Beriberi is undoubtedly the constant hypertrophy of the Adrenals. McCarrison (2) was the first to note this extensive hypertrophy of the adrenals in pigeons fed on polished rice. Unfortunately in examining sections of the glands he failed to use any special stains for fat and thus came to the conclusion that the hypertrophy was a true one only so far as the chromaffin tissues were concerned. By injecting emulsions of the glands into the jugular vein of a sheep and noting the rise in blood pressure, he demonstrated what appeared to be a definite increase in the adrenalin contents of glands removed from Beriberic pigeons.

In the present series of experiments it has been shown that this adrenal hypertrophy is more probably due to the increased amount of lipoid stored in the cortical cells, since the cortical cells appear to form a greater proportion of the section in diseased than in healthy birds - a condition in fact clearly shown by some of McCarrison's figures.

If, however, a bird suffering from paralytic symptoms be given a curative dose of Yeast and then killed some six hours afterwards, a striking change will be noted in the cortical cells. The lipoid content has decreased to a remarkable degree, and is now usually less than that normally present in controls.

In discussing human Beriberi it has also been shown that there is an increase in lipoid material, which is now found both in cortical and medullary cells. It would seem that the medullary cells are able to a certain extent to take on the function of the cortical cells. The reverse has also been shown, for Cramer (22) by stimulating artificially the formation of Adrenalin was able by fixing the glands in Osmic Acid vapour and then dissolving out the lipoid with turpentine to show the presence of Adrenalin granules both in cortical and medullary cells.

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From the chemical standpoint, the material in the adrenal cortex appears to consist of lecithin, cholesterolin and fatty acid.

When Water Soluble B factor is administered to a bird with Beriberic paralysis there are thus three changes which occur with remarkable rapidity:-

- (1) the disappearance of the paralysis.
- (2) the reappearance of the Nissl granules.
- (3) the reduction in the lipoid content of the adrenal cortex. These changes would appear to be intimately related one with another and with the administration of Water Soluble B factor.

To obtain an explanation of this correlation it is necessary to enquire into the object of the increase of lipoid in the Adrenal Cortex, from which there arises the further question as to the function of the Adrenal Cortex in the body. In the main the adrenal cortex would appear to be associated with two sets of organs, the nervous system and the genital system. That the cortical lipoid serves as a reservoir of food material both for the developing and the adult nervous system is shown by the fact that there is a correspondence in size between the brain and the adrenal cortex in the animal series, while in anencephalous monsters there is a diminution in the size of the cortex. Quite recently also Prior & Jones (45) have pointed out that in epileptics one finds in a high percentage of cases degenerative changes in the cortex.

Furthermore the adrenal cortex regulates the supply of lipoid to the reproductive organs. Mott (46) in a recent paper on the testicles in dementia praecox has shown that phosphorised lipoids, since they are essential to the maintenance of spermatogenesis, almost certainly constitute one of the precursors of animal nucleic acid - an assumption supported by the following facts:-

- (1) During the breeding season the salmon takes no food. At the same time its genital organs increase enormously in size and it produces millions of spermatozoa, the heads of which, as Kossel (47) showed are composed of nucleic acid and a protamine "salmin". The protamine comes from the muscles, while in the breeding season the blood is found to contain a large excess of phosphorised lipoid, which is probably used therefore in producing the heads of the countless spermatozoa.
- (2) In the fresh egg, neither nucleoproteins nor pentoses are present while purin bases are only to be found in small traces. During development, however, these substances increase in amount, having been formed from the food material present in the egg, i.e. protein and phosphorised lipoid. In the fresh egg, as Plimmer and Scott (48) have shown, 65% of the total phosphorus is present in the form of lipoid while in the chick only/

only 20% is so combined. If then phosphorised lipid forms a nucleic acid precursor in the testicle as shown by Mott; it seems more than probable that it may also form a nucleic acid precursor in the central nervous system.

It may, however, be urged that the adrenal cortex hypertrophies in order to protect the myelin in the medullary sheaths of the nerves. As was previously pointed out the only histological change in the central nervous system constantly associated with Beriberic paralysis is the disappearance of the Nissl granules. In one case of a pigeon with Beriberic paralysis, though no definite changes could be found in the myelin, the adrenals were markedly enlarged. All attempts to correlate the degree of degeneration in the nerve fibres with the degree of hypertrophy in the adrenals have failed. It has been suggested on the other hand, that if the lipid passes from the adrenal Cortex to the nerve cells then it should be possible to correlate the hypertrophy of the adrenal cortex with the degree of degeneration in the nerve cells. This does not, however, follow.

When a bird dies of Beriberi it does so eventually owing to failure of the respiratory and cardiac functions, due to interference probably with the cardiac and respiratory centres of the medulla. Death may occur a few hours or days after the onset of paralysis, but the end result is the same so far as the nervous system is concerned. Perhaps a military simile will help to make clear this point. We may suppose that troops are besieged in a fort by the enemy. Some miles away a number of troops are collected in reserve but they cannot be taken up to relieve the fort because no transport is available. Obviously the fort will fall when a certain number of its garrison have been killed. It matters little to the garrison whether there are a thousand or ten thousand men in reserve.

To summarise then, it would appear that the changes in the nerve cells in Beriberi are indicative of nuclear starvation. Yet in Beriberi we find stored up in excess in the adrenals one of the substances which are probably essential to the formation of Nucleic Acid. When Water Soluble B factor is administered, there is on the one hand a removal of the excess of lipid in the adrenal cortex, and on the other hand an end to nuclear starvation.

Provisionally then we may formulate the hypothesis that Water Soluble B factor forms an essential link in the conversion into Nucleic Acid of certain Nucleic Acid precursors.

When the tissue content of Water Soluble B factor falls below a certain minimum the phosphorised lipid instead of passing on to the central nervous system is immobilised in the adrenal cortex; the central nervous system begins to run short of nuclear material and a functional paralysis occurs. If the nuclear shortage becomes very acute, the essential nerve centres/



centres in the medulla are at length affected and death ensues, but if Water Soluble B factor is given in time, the lipoid of the adrenal cortex is liberated, the nuclear starvation of the central nervous system disappears and the paralytic bird is restored to health with dramatic rapidity.

If the hypothesis which we have suggested be correct several facts can be viewed in a new light. Thus it becomes apparent why muscular activity, by using up nuclear material in the central nervous system, as evidenced by chromatolysis, causes a shortening of the incubation period of Beriberi; why in fact any condition such as growth, entailing an increased demand for nuclear material should more rapidly use up the available supply of Water Soluble B factor, while starvation and hibernation in which nuclear activity is reduced to a minimum prolong the incubation period. The results of total extirpation of the adrenals are also of fresh interest in this connection.

In 1892 Abelous and Langlois (49) noted the effects of extirpation of the adrenals in frogs.

The first symptoms appeared 24-36 hours after the operation and consisted in a progressive paralysis, beginning in the hind limbs, then passing on to the front limbs until the animal became completely inert. The respirations were slowed, asthenia was pronounced and the animal died. If stimulated from time to time so as to provoke movements, paralysis appeared more rapidly and death more quickly. The more active the chemical processes in the animal, e.g. in summer frogs as compared with winter frogs, the more quickly did death supervene.

Gourfein (50) performed similar experiments with pigeons which died with practically the same symptoms in from 4-24 hours after extirpation of the glands, while Donetti (51) found changes in the nerve cells of guinea pigs and rabbits, more especially in the medulla, the nuclei being vascular, eccentric or even totally absent. The similarity of these findings to the clinical symptoms and histological changes in the central nervous system of Beriberi is rather remarkable. It would seem that in these cases of extirpation of the adrenals, the central nervous system suffers because there is no adrenal cortex from which it can obtain its necessary supply of lipoid for partial conversion into nucleic acid, while in Beriberi the lipoids are present in the adrenal cortex, but are immobilised in the absence of Water Soluble B factor. In both instances the central nervous system suffers in a similar manner.

It is well known that patients with Addison's disease are rapidly fatigued. Now in many cases of Addison's disease the adrenal cortex and medulla are both affected and hence the cause of the fatigue may be due not so much to the loss of Adrenalin as to the fact that the Central Nervous System is not getting its full supply of lipoid from the adrenal cortex.

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It must not be forgotten, however, that a certain degree of hypertrophy occurs in the adrenal cortex of animals dying of inanition even when Water Soluble B factor is present in the diet. In starvation, it will be remembered, the brain undergoes little or no atrophy; it is maintained at the expense of organs less essential to the vital economy. If then the adrenal cortex maintains the normal nutrition of the central nervous system, it is not surprising that with supplies at a low ebb, the adrenal should as it were "corner" as much lipoid as it can obtain. Thus the testicles and ovaries, both great consumers of lipoid, undergo atrophy both in Beriberi and inanition. Since the genital organs are not absolutely essential to the life of the individual, their nutrition goes to the wall when the integrity of the central nervous system is at stake.

The main function of the adrenal medulla appears to be the production of adrenalin which passing into the blood stream controls blood pressure, acts on the peripheral neuro-muscular elements in the arterioles, promotes the activity of the skeletal muscles and in association with the other endocrine glands plays a part in the regulation of Carbohydrate metabolism. McCarrison (2) claims to have demonstrated an increase in the adrenalin content of glands removed from pigeons suffering from Beriberi. In the present instance physiological methods were not employed for estimating the adrenalin content, but by histological methods, e.g. fixation of the glands in Osmic acid vapour or potassium bichromate solution, no increase in the adrenalin content could be demonstrated. It must also be remembered that the load of Adrenalin in a gland represents merely the balance of formation and excretion but gives no indication of the activity of the gland; this latter is determined by the rapidity with which the adrenalin is built up and given off. The finding of an excess of adrenalin in the glands is therefore open to the interpretation of a diminished output of adrenalin into the blood stream.

McCarrison, however, goes further than the mere finding of an excess of adrenalin in the glands, for he believes that this excess is the main factor in producing oedema, which oedema, in experimental birds at any rate, occurs in the form of hydropericardium; he also believes that the excess of adrenalin is a direct result of the lack of Water Soluble B factor in the diet. The evidence brought forward in support of this contention is as follows:- In a first series of adult pigeons fed on polished rice, 70% had oedema in some form or another; one hundred per cent of cases having oedema had adrenals of 147 mgs. per kilo or over.

In a second series of young pigeons oedema was present in 75%; the average weight of the adrenals in "dry" beriberi was 127 mgs. in "wet" beriberi 196 mgs.

also Yamagiwa (52) is quoted - In 1899 he reported changes in/



in the blood vessels in Beriberi particularly in the media of the arterioles. In preparations of nerves, muscles and kidneys he always found the walls of the arterioles thickened and the lumen narrowed, with the result that he believed the essence of Beriberi to consist in the increased resistance which occurs in the systemic and pulmonary circulations as a result of the contraction of the arterioles.

It is somewhat doubtful whether an entirely good case has been made out for this close connection between the increase of Adrenalin and the presence of Oedema. The following facts would seem to disprove the relationship altogether:-

(1) The main factor in the production of adrenal hypertrophy is not so much an increase in the medullary as in the cortical cells, which are concerned with the storage of lipoid.

(2) Although adrenal hypertrophy was present in every instance in the present series of fowls and pigeons, oedema was not of frequent occurrence in the experimental birds. The fowl with the biggest adrenals - 401 mgs. - had no sign of oedema anywhere. There would thus seem to be little relationship between the size of the adrenals and the occurrence of oedema in birds.

In fact in his more recent paper on Beriberi in monkeys McCarrison (29) does not lay nearly so much emphasis on this correlation.

(3) Since Yamagiwa recorded his findings in 1899, many observers have examined post-mortem material from cases of human Beriberi, but none have recorded any hypertrophy of the muscular coats of the arterioles.

(4) Although possibly Adrenalin may have some slight action on the capillaries, its main pressor action is certainly on the neuro-muscular elements in the walls of the arterioles. It is difficult to understand how the action of adrenalin on the arterioles could produce in the capillaries a pressure sufficient to lead to increased transudation and oedema.

(5) Clinically patients suffering from Beriberi show no evidence of an increased blood pressure at any time. The reverse is in fact the case and the blood pressure is often abnormally low.

(6) Arteriosclerosis is said to be produced in rabbits by the repeated administration of adrenalin (Josué (53)). Arteriosclerosis is not especially marked in human beings dying from Beriberi nor has it been recorded in birds fed on polished rice.

(7) In 1917 on clinical grounds alone I was led to recommend the administration of adrenalin in cases of human Beriberi suffering from marked asthenia. So far from producing any bad effects or any increase in the oedema the reverse was the case (Findlay (33)).

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It will thus be seen that though possibly an increased amount of adrenalin may pass into the circulation in an attempt to maintain normal blood pressure and muscular tone, nevertheless there is no evidence to connect such an increase with the adrenal hypertrophy or to show that the secretion of an excess of adrenalin is ever capable of producing oedema.

The question therefore remains as to what is the cause of Oedema in Beriberi. Any theory to be entirely satisfactory must adequately explain why oedema is more common in man than in birds suffering from Beriberi, why some cases of human Beriberi never exhibit oedema, while others e.g. those occurring on board ship are always of the "Wet" type.

Although a very large amount of research has been carried out on the cause of Oedema no very satisfactory explanation has as yet been obtained. It is recognised however, that in any given case of Oedema several factors may contribute to its production.

In the case of Beriberi it would seem that the chief factor in the causation of Oedema is the presence of degenerative changes in the endothelial lining of the capillaries.

This endothelial degeneration may be produced in several ways:-

- (1) The direct action on the endothelium of lack of Water Soluble B factor.
- (2) The possible presence of toxic substances of the histamine type in the blood.
- (3) The prolonged dilatation and hypertrophy of the right heart.
- (4) Interference with the vasomotor nerve supply of the arterioles.

It will be necessary to discuss these points rather more fully.

(1) In recent years it has come to be recognised that the endothelial cells of the capillaries have a definite secretory action and do not act merely as a passive membrane. In discussing the histological findings it has been pointed out that in all organs it is the secretory cells which are the most affected and which show most the evidences of nuclear starvation. It would be surprising, therefore, if the functional activity of the capillary endothelium were not impaired by the lack of Water Soluble B factor.

(2) The recent experiments of Dale, Richards & Laidlaw (54) and (55) on the effects of histamine have emphasised the fact that the capillary endothelium is very sensitive to the effects of toxic substances in the blood. In Beriberi/is not improbable that certain bodies akin to histamine pass into the general circulation.

(3) Recently/



(3) ~~Recently~~ Bolton (56) has produced experimental œdema in animals by tying off a portion of the pericardial sac, with the result that the remainder is too small to allow of the heart's free expansion. Its diastole is interfered with and there results first a marked dilatation of the great veins and later œdema. Observations on the arterial and venous blood pressures negative the suggestion that this is caused by an increase of capillary pressure. The œdema would appear to be due rather to increased permeability of the capillaries, brought about by deficient oxygenation of the blood and as a result impaired nutrition of the capillary wall. The original cause of the œdema in these experiments was the reduction in size of the pericardial sac: now as the pericardial membrane cannot stretch indefinitely it follows that a reduction in the size of the pericardial sac may also be caused by prolonged dilatation and hypertrophy of the right heart.

In human Beriberi œdema frequently occurs before any signs of peripheral neuritis are noted. But even before the occurrence of œdema it is possible by careful percussion to establish the presence of marked dilatation of the right heart. My attention was first directed to this point by Skopophoulos of Port Said, who has had a very large experience of ship Beriberi. As a result, while examining the crew of a ship in which cases of Beriberi were occurring, I was able to predict successfully that in two cases œdema would occur in a few days.

While on active service in Egypt, I received cases of Beriberi from sixteen of H.M. Ships stationed in the Red Sea and Indian Ocean. I was able to determine that in every ship in which cases of Beriberi had occurred a few men had complained of œdema of the feet, breathlessness and palpitation of the heart. Rest in bed and a change of diet rapidly cured these patients, who at no time exhibited any signs of peripheral neuritis. Marshall (59) has recorded similar cases. All cases of Beriberi occurring on board ship are of the œdematous type and all have marked dilatation of the right heart. Sailors as distinguished from landsmen are rather liable to dilated hearts due to boat pulling, hauling on ropes and stoking. Beriberi, however, is not always associated with œdema; certain cases may never show signs of œdema at any time. It is curious, however, that cases of Beriberi in infants are always of the œdematous type and in infants the right heart is always dilated. In the adult, enlargement of the heart down and to the right is very common, but is by no means a constant post-mortem finding. In birds œdema is not common, neither is dilatation of the right heart. In the present series the two fowls with the largest amount of fluid in the pericardial cavity also had marked dilatation of <sup>the</sup> whole of the right heart.

(4) In describing the nerve lesions in Beriberi it was pointed/

pointed out that there is a certain capriciousness in the nerves affected. Ellis (57) as the result of a large number of post-mortems came to the conclusion that in dry Beriberi the motor nerves were the most affected while in wet Beriberi the sympathetic nerves were more frequently attacked.

In this connection an old experiment of Ranvier (58) is of interest. He ligatured the inferior vena cava of a dog and then sectioned the sciatic nerve : œdema occurred. If, however, only the motor fibres in the sciatic were cut, no œdema followed, but on cutting the vasomotor fibres also œdema promptly occurred.

According to this theory then Oedema in Beriberi is essentially due to impairment of the capillary endothelium, this impairment being due to the combined action of various factors.

Since Beriberi in man and animals is not necessarily associated with œdema, it is strictly comparable to a number of diseases such as dysentery, malaria or ankylostomiasis in which œdema may occur and in which there is a definite toxic action on the capillary endothelium. The condition known as "War Oedema" must also probably be grouped with these diseases, the impairment of the capillary endothelium being due in this instance to chronic inanition.

(b) The Pituitary undergoes little or no change either in inanition or in beriberi, except that of course certain of the nuclei show characteristic changes indicative of degeneration. The functions of the Pituitary are apparently of such importance that they are maintained at the expense of other less necessary organs.

(c) The Thyroid also undergoes very little atrophy either in inanition or beriberi. There is perhaps a rather greater tendency to the conversion of thyroid into parathyroid like tissue in beriberi, but this change is often quite well seen in the thyroid of healthy birds.

(d) The Thymus plays a part in normal metabolism which is still but imperfectly understood. In birds there is a total disappearance of the thymus both in beriberi and in inanition even when a plentiful supply of Water Soluble B factor is present in the diet. That the atrophy really is complete is shown by the following experiment. One pigeon which had developed paralytic symptoms on a rice dietary was relieved by the administration of Yeast and thereafter was fed on mixed grains for six weeks. Histologically, on killing the bird, the organs appeared normal, the testicles showed active spermatogenesis but there was no thymus present. It is doubtful, therefore, if there is any correlation between the thymus and testicular activity in the male pigeon.

In Infantile Beriberi, Andrews (30) states that the thymus is always present and may even show an increase in/



in cellular elements. From the chemical point of view the thymus contains a large quantity of nucleoprotein and Water Soluble B factor. The liberation of this Water Soluble B factor may perhaps delay very slightly the onset of Beriberi, but it seems more probable that the thymus forms a reserve supply of food apart altogether from its Water Soluble B content. Atrophy of the thymus was recognised by Wharton in the seventeenth century as a concomitant of exhausting or wasting disease.

(e) The Spleen, like the thymus, shows a very considerable degree of atrophy both in Avian Beriberi and in acute and chronic inanition. In human Beriberi there is also atrophy to a certain degree. Probably the lymphoid tissues of the body are all used up as food material when the more essential tissues run short of nourishment. The Splenic atrophy cannot therefore be looked upon as a specific result of the lack of Water Soluble B factor.

With the exception then of the Thymus and Spleen the Endocrine glands are less affected than the other organs of the body. As regulators of Metabolism together with the nervous system their integrity is maintained till the last possible moment both in Beriberi and inanition.

- (3) The Genital Organs have already been mentioned in connection with the rôle of the Adrenal Cortex in the metabolism of Water Soluble B factor. It would seem that when the nutrition of the central nervous system is in danger, whether through lack of food material as in starvation or because the necessary food material is immobilised for want of Water Soluble B factor, the genital organs promptly return to their condition prior to puberty - spermatogenesis and ovulation cease. The undifferentiated cells found in the testicles of beriberic pigeons are, however, quite capable of regeneration when the necessary food stuffs are administered. In one instance active spermatogenesis was found in the testicle of a pigeon which six weeks previously had suffered from Beriberi. The relationship existing between the food supply and fertility is one of immense importance. For the production of healthy offspring the mother requires a plentiful supply of Water Soluble B factor and a plentiful supply of phosphorised lipoid. The supply of lipoid may be limited in two ways (1) by an insufficient supply of the basic food stuffs, resulting in a deficient synthesis of lipoid in the body and (2) by a diet deficient in Water Soluble B factor, since in this condition the lipoid is immobilised in the adrenal cortex. The second essential necessary for fertility - a plentiful supply of Water Soluble B factor - can only be obtained by a diet rich in this factor. In birds fed on polished rice there is obviously/

obviously a deficiency both in Water Soluble B factor and in the basic food stuffs necessary for the synthesis of lipoid.

The effects of a copious food supply on fertility have been known since the days of Aristotle, who commented on the great fertility of domestic as compared with wild sheep. On the other hand it is well known to breeders that an excessive diet of sugar or molasses when given to cattle produces sterility. This is of some interest when it is remembered that an excess of Carbohydrate in the diet seems to use up the available supply of Water Soluble B factor. In thinking over the relationship of Accessory food factors to fertility one is tempted to hazard the suggestion that malaria in man is really due to the existence of Accessory food factors. The male Anopheline is content to live on fruits and their juices - a diet not too rich in Accessory food factors, but the female, with the burden of pregnancy upon her, must obtain from somewhere a more plentiful supply of these factors. She finds them in the blood of man.

Many interesting clinical facts have been correlated with the absence of Water Soluble B factor from the diet. Thus Vedder (14) has noted ~~that fact~~ that not infrequently women develop Beriberi while pregnant owing to the large amount of Water Soluble B factor required by the developing foetus. Women with Beriberi cease to menstruate, while I myself have found that in men one of the earliest symptoms of Beriberi is the cessation of seminal emissions. McCarrison (2) has suggested that the "War Amenorrhoea" met with on the continent is attributable to the absence of Accessory food factors from the diet. In this instance, however, the chronic insufficiency of the basic food substances, fats, carbohydrates and amino acids may also have played a part.

4. The Kidneys. Beyond the parenchymatous degeneration of the cells of the convoluted tubules and the evidence of nuclear starvation, the changes in the kidneys are not very marked in Beriberi. This fact is probably connected with the rarity of Albuminuria in human Beriberi. It is of some interest to note that according to one theory, oedema is produced by the imperviousness of the kidney to chlorides, and their retention in the fluids of the body. The administration of chlorides in large doses to fowls suffering from beriberi does not however lead to the production of oedema. Since hyperplasia of the adrenals has been associated with certain forms of Nephritis an attempt was made to correlate the size of the adrenals with the degree of degeneration in the kidneys, but without success.
5. The Heart. The possible rôle of the heart and blood vessels in leading to the production of oedema has already/



already been discussed.

There remains, however, the curious fact that while hypertrophy of the right heart is very common in human Beriberi, in Avian beriberi it is never seen. Under normal conditions the heart muscle contains a fairly large quantity of Water Soluble B factor, which would thus appear to be essential to its activity. When the available supply of Water Soluble factor runs short the heart muscle is necessarily weakened with the result that the heart is unable to empty itself satisfactorily.

This would explain the dilatation of the heart in human and avian beriberi.

The hypertrophy in human beriberi may be due to the fact as noted by Paton (60) that in birds only the auricle receives sympathetic accelerator fibres, while in man these fibres are distributed to both auricle and ventricle. In other words adrenalin plays a part in maintaining the normal nutrition of both ventricle and auricle in man, while in birds only the auricle is so stimulated. In man the whole pathological process set up by lack of Water Soluble B factor is more chronic than in birds. Hence the human heart does not feel the lack of the Water Soluble B factor so rapidly and before the shortage becomes acute is able to hypertrophy.

## 6. The Alimentary System.

(a) The Alimentary Canal exhibits very similar changes in birds suffering from Beriberi and inanition. In both cases there is congestion of the capillaries, atrophy of the muscular layers and lining epithelium, disappearance of the lymphoid masses and a cessation of all secretory activities.

The disappearance of the lymphoid masses is doubtless part of the generalised process whereby all lymphoid tissue, as in the thymus and spleen, is used up as a reserve food supply.

In man this atrophy of the lymphoid tissues is not nearly so well marked.

In one important respect, however, the changes in the alimentary canal in Avian Beriberi differ from those seen in inanition, for in the former condition the nerve plexuses are degenerated. These plexuses regulate both the peristaltic and swaying movements of the intestine. Their impairment in cases receiving an insufficient supply of Water Soluble B factor may possibly be one of the factors leading to chronic intestinal stasis.

The frequency of a generalised infection with *B. coli* in birds suffering from Beriberi and inanition would seem to indicate that the resisting power of the intestinal wall has been seriously impaired.

(b) The Pancreas shows similar degenerative changes both/

both in Beriberi and inanition; they would thus seem to be due rather to the effects of inanition than to the specific lack of Water Soluble B factor. In fowls which had died from acute inanition there appeared to be a very slight increase in the number of the islets of Langerhans as described by Vincent and Thompson (61). In pigeons dying of chronic inanition and in birds dying of Beriberi no such increase was noticeable.

(c) The Liver changes are practically identical in Beriberi and inanition, though in the former condition the evidences of nuclear starvation are of course well marked.

### CONCLUSIONS.

(1) The effects produced in birds by an exclusive diet of polished rice and by inanition are very similar.

(2) The specific effect of lack of Water Soluble B factor is Nuclear Starvation.

(3) Nuclear Starvation, leading to a functional impairment, is seen in every organ of the body.

(4) In the Nervous System, Nuclear starvation produces clinically a functional paralysis, histologically degeneration of the myelin sheaths and chromatolysis.

(5) In the Adrenals the lack of Water Soluble B factor leads to a storing up of lipid in the cortical cells.

(6) The administration of Water Soluble B factor to a bird with Beriberic paralysis cures the paralysis; at the same time the Nissl granules reappear in the nerve cells and the excess of lipid disappears from the Adrenals.

(7) Under normal circumstances phosphorised lipid acts as a precursor of Nucleic Acid.

(8) In the absence of Water Soluble B factor, phosphorised lipid cannot go to form Nucleic Acid.

(9) Water Soluble B factor would appear to act as a link by means of which the various substances forming the complex molecule of Nucleic Acid are held together, being essential to the synthesis of Nucleic Acid in the animal body.



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## PART 111.

## BIOCHEMICAL EXPERIMENTS.

At the present time two theories are held as to the possible mode of action of the Accessory food factors. To some it would seem that the function of these factors is essentially catalytic, a view which found expression as far back as 1911 (Hopkins (1)). To others, however, the accessory food factors would appear to be indispensable tissue elements in the structural sense; exceptional in that like certain of the Amino-acids they cannot be synthesised by the animal tissues.

It is unlikely that the Accessory food factors can be enzymes in the commonly accepted sense of the word. The animal body would appear to be capable of producing enzymes in immense variety; that it cannot produce substances so essential to the vital economy as Water Soluble B and fat Soluble A would be strange if these factors were true enzymes. In addition Water Soluble B factor is comparatively thermo-stable; when exposed to a temperature of 100°C. destruction only occurs very slowly, though more rapidly at 120°C; most enzymes on the other hand are destroyed, at a temperature of 65°C-75°C, though some oxidases it is true can be heated to 100°C and Trypsin in acid solution can be boiled for a considerable time.

McCollum (2) and others are strongly of opinion that the Accessory food factors are "food complexes, necessary for all the living cells of the body", since all the available evidence indicates that both Fat soluble A and Water Soluble B factors are never failing constituents of the cells of both animal and plant tissues. The evidence derived from the present experiments certainly indicates that the lack of Water Soluble B factor is felt by all the cells of the body.

If then a satisfactory explanation of the function of Water Soluble B factor is to be obtained, two conditions must be fulfilled.

- (1) The function must be common to all cells.
- (2) The function must be of greater importance in youth than in adult life, for Water Soluble B factor is essential to growth, while young animals develop symptoms referable to its loss more quickly than adults.

A function common to all cells might well be connected with the cell nucleus, while in regard to the second condition youth as distinguished from adult life is essentially a period of growth, i.e. a period of rapid cell division. Now cell division is initiated by the activity/



activity of the nucleus. Thus once more in connection with Water Soluble B factor we are led back to a consideration of the cell nucleus. Various attempts have been made from time to time to isolate Water Soluble B factor in a state of purity, but hitherto without success.

In 1911 Funk (3), after certain preliminary investigations with Cooper (4), succeeded in preparing a crystalline substance from an alcoholic extract of rice polishings. This substance which was curative for pigeons belonged to the class of pyrimidine bases and was given the formula  $C_{17}H_{20}N_2O_5$ .

More recent work, however, seems to suggest that this nitrogenous base was merely contaminated with traces of the Water Soluble B factor (Barger (5) Drummond and Funk (6)).

Later Eadie, Evans, Moore, Simpson and Webster (7) prepared an organic base from yeast. This substance also belonged to the pyrimidine group and had a formula  $C_{17}H_{17}N_2O_5$ .

Water Soluble B factor behaves in a somewhat interesting way towards certain adsorbents. Thus Chamberlain and Vedder (8) found that it is adsorbed from solution by bone black, while Seidell (9) showed that it was adsorbed quantitatively by a pure preparation of fuller's earth, called Lloyd's reagent. By employing this reagent Williams and Seidell (10) attempted to isolate Water Soluble B factor from autolysed yeast. A substance identical with adenine was obtained, which at first was very active but later lost all activity. Pure adenine from yeast is totally inactive (Voegtlin and White (11) and Harden & Zilva (12)). Probably the Water Soluble B factor was associated with adenine as an adsorption product, much in the same way as it is found to be associated with nicotinic acid (Barger and Ewins, quoted by Barger (5)). Hofmeister (13) has recently isolated a very active substance belonging to the pyrimidine series to which has been given the formula  $C_5H_{11}NO_2$ .

These unsuccessful attempts to isolate Water Soluble B factor have been discussed in some detail because they serve to emphasise two points of importance; - the readiness with which the factor forms adsorption compounds and the frequency with which it has been found associated with substances, which are formed as hydrolytic products either of Plant or Animal Nucleic Acid.

The question naturally arises as to whether Water Soluble B factor is actually Nucleic Acid itself.

This suggestion was first made by Schaumann in 1908 (14). To test this, hypothesis Grifns (15) isolated the nucleins in an impure state from the bean *Phaseolus radiatus* - Katjang idjo. The Nucleins derived from Katjang idjo had no protective action against Beriberi, but an infusion/

infusion of katjang idjo with its nucleins removed was quite active. These results were confirmed by de Haan (16). From this it would appear that Nucleic Acid does not contain Water Soluble B factor. It must, however, be remembered that the methods in use for isolating nucleins are somewhat drastic, involving as they do prolonged boiling with fixed alkalies. Vedder & Clark (17) have shown that the activity of Funk's base is rapidly destroyed by treatment with such alkalies.

On the other hand substances particularly rich in Plant or Animal Nucleic Acid are also particularly rich in Water Soluble B factor, e.g. Yeast and Thymus; but certain other substances, e.g. Milk and Yolk of egg, while containing no Nucleic Acid are rich in Water Soluble B factor. These food stuffs are, however, essentially Nuclein formers.

In order to determine whether there is any quantitative relation between the Nucleic Acid content and the Water Soluble B content of the tissues, certain experiments were carried out with the organs of the Ox. This animal was selected because the Water Soluble B content of its organs has been worked out both by Cooper (18) and Chick and Hume (19) by estimating the daily ration of the particular organ necessary to prevent Beriberi when added to diet consisting solely of polished rice. The minimum daily ration of the dried organ which must be added to such a diet of polished rice to prevent the onset of Beriberi in a pigeon of 300 to 400 grms weight is as follows:-

Organ of Ox.	Dry Weight (gram).
Liver	0.9
Brain	1.2
Heart	1.7
Muscle	5.0.

If then the Water Soluble B content of Ox liver be taken as equal to 100, the relative Water Soluble B value of the organs is

Organ	Value
Liver	100
Brain	75
Heart	52.9
Muscle	18

For the estimation of Nucleic Acid the fresh tissues of the Ox were employed. The method of extraction recommended by Jones (20) was employed. Briefly this method is as follows:-

1 kg of the organ (liver, brain, heart, muscle) finely trimmed and minced is added to a boiling mixture of 2 litres of Water, 100 gram of Sodium acetate and 33 gram of Caustic Soda. The tissue dissolves to form a pale brown fluid. The vessel containing the fluid is/



is placed on a briskly boiling water bath and allowed to remain there with occasional stirring for two hours. The alkaline fluid is then rendered just acid with 50 per cent acetic acid, brought rapidly to the boil and filtered with a hot water funnel.

The filtrate and washings are evaporated on a water bath to about 750 c.cs and while warm the concentrated solution is slowly poured into 1 litre of 95 per cent alcohol. On standing overnight the precipitated sodium nucleate settles ~~sharply~~ to a spongy white mass from which the bulk of brown alcoholic fluid can be sharply decanted and the remainder pressed out with a spatula leaving the material in one cohesive mass.

The substance is washed by decantation in turn with 80 per cent and 95 per cent alcohol and, after pressing out the last wash fluid as far as possible, is transferred ~~to~~ to a flask with 300 c.c. of hot water and heated on a water bath.

In half an hour or less, insoluble phosphates will collect leaving a perfectly transparent interstitial fluid which is treated with 10 c.c of 20 per cent caustic soda to lower the viscosity and filtered with a hot water funnel. The perfectly transparent yellow filtrate is again acidified with acetic acid and poured into 700 c.c of 95 per cent alcohol when sodium nucleate is precipitated. This can be washed by decantation as before with alcohol and ground in a mortar with absolute alcohol until it has crumbled to a fine white powder. The material is finally washed to a filter with absolute alcohol and allowed to dry in a sulphuric acid desiccator. The product is a soluble sodium salt of nucleic acid, but is generally referred to as animal nucleic acid. The actual weight of nucleic acid obtained from the various organs was as follows, the average of five readings being taken:-

Organs	Wt. of Nucleic Acid in grms per Kilo of Organ.
Liver	30.24
Brain	28.46
Heart	18.19
Muscle	6.26

There was probably a certain amount of mechanical loss of Nucleic Acid due to the processes of extraction. In order to compare the Nucleic Acid content with the Water Soluble B content of the organs it is obvious that the amount of Nucleic acid content in the dry weight of the organ must be compared. Taking the Nucleic acid content of the liver as 100, the following values are obtained:-

Organ	Value	Approx. Water content
Liver	100	70
Brain	72.2	77
Heart	50.9	75
Muscle	17.2	75

When due allowance is made for the very considerable experimental error which may occur both in the estimation of Water Soluble B factor and of Nucleic Acid, it will be seen that there is a somewhat surprising parallelism between the values obtained for Water Soluble B factor and for Nucleic Acid. This would seem to suggest that in the tissues of the adult the amount of Water Soluble B is proportional to the amount of Nucleic Acid present.

The next step was obviously to investigate the Nucleic Acid content of the pigeon's organs. For this purpose the brain and the liver were selected, the method used for the extraction of Nucleic Acid being the same as that previously described. Observations were made on the liver and brain of (1) normal pigeons (2) pigeons suffering from beriberi (3) pigeons with beriberi eight hours after the administration of a curative dose of yeast. The values obtained are recorded in grms per kilo of the organ and are shown below:-

	Normal pigeon	Beriberi pigeon	Beriberi pigeon cured with yeast
Nucleic Acid in Liver	29.25	19.40	21.50
Nucleic Acid in Brain	27.75	23.45	26.08

It will thus be seen that the diet of polished rice induces a considerable loss of Nucleic Acid in the liver - 30.3%, while in the brain there is only a loss of 15.5%. The administration of yeast, however, causes a considerable increase in the Nucleic Acid content of the brain, but only a very slight increase in the Nucleic Acid content of the liver.

Since, however, there would appear to be some connection between the Nucleic Acid content and the Water Soluble B content of the tissues, it follows from this last experiment that even in the tissues of birds suffering from Beriberi there should be an appreciable amount of Water Soluble B factor.

That this is the case is shown by the following experiment:-

Five healthy hens were placed on a diet of polished rice and in addition were given on alternate days 1 gm of the organs of a pigeon dying of Beriberi. The experiment continued for fifty days.

No 1	received	ad libitum	polished rice	+ 1 gm	of Brain of beriberic pigeon.
No 2	"	"	"	" + 1 gm	of Kidney of beriberic pigeon.
No 3	"	"	"	" + 1 gm	of Muscle of beriberic pigeon.
No 4	"	"	"	" + 1 gm	of Heart Muscle of beriberic pigeon.
No.5	"	"	"	" + 1 gm	of Liver of beriberic pigeon



At the end of the experiment none of the birds showed evidence of Beriberic paralysis and all appeared in good health.

No.1	had increased its weight by 24.3%	of its weight at the beginning of the experiment.
No.2	" " " " "	15.0% " " "
No.3	" " " " "	11.9% " " "
No.4	" " " " "	9.9% " " "
No.5	" decreased " " "	2.4% " " "

On examining the tissues of the various birds histologically no evidence of Beriberi could be found in any, though in No. 5 there was some evidence of cloudy swelling in the parenchymatous cells of the liver and kidney. It may, therefore, be concluded that the organs of birds suffering from Beriberi have a definitely protective influence against the development of Beriberi, in other words they contain an appreciable amount of Water Soluble B factor. Although the number of experimental birds is insufficient to warrant any definite statement, it is curious that the bird with the greatest increase in weight was fed on brain, the bird with a decrease in weight on liver. This experiment would suggest that as long as the tissues contain any Nucleic Acid they also contain Water Soluble B factor.

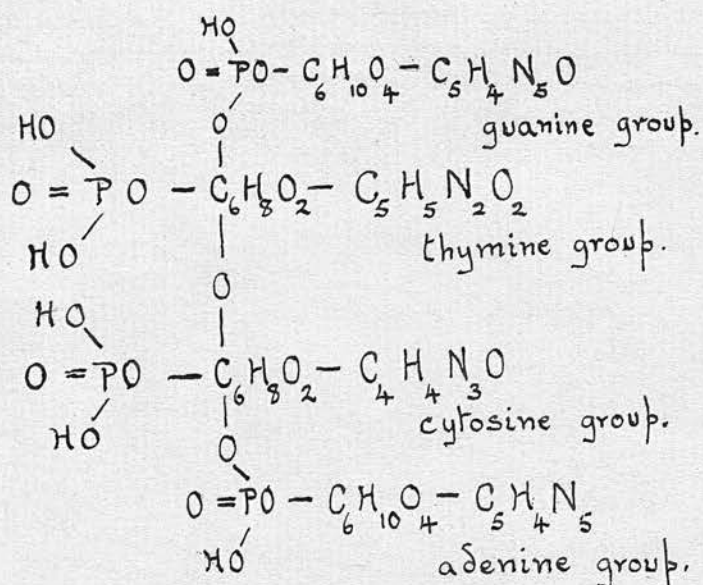
#### CONCLUSIONS.

- (1) The Nucleic Acid content and the Water Soluble B factor content of the organs of the Ox are in some way related quantitatively.
- (2) As contrasted with normal birds, the liver and brain of Beriberic birds show a decrease in the Nucleic Acid content, more marked in the liver than in the brain.
- (3) The administration of Water Soluble B factor to a bird with Beriberic paralysis is followed by a marked rise in the Nucleic Acid content of the brain, by a slight rise in the Nucleic Acid content of the liver.
- (4) The organs of birds dying from Beriberi still contain an appreciable amount of Water Soluble B factor.

## GENERAL CONCLUSIONS.

As a result of the experiments on Avian Beriberi, it is now possible to formulate the possible rôle of Water Soluble B factor in the vital economy. This rôle would appear to be intimately connected with Nucleic Acid.

It is now agreed that from whatever animal tissue it is obtained, Nucleic Acid always has the same composition - in other words, there is only one Animal Nucleic Acid. The composition of this Nucleic Acid is usually represented by the following formula:-



Though complicated and of course liable to revision, this formula shows that Animal Nucleic Acid is made up of Phosphoric Acid, a hexose group, two purin derivatives, adenine and guanine, and two pyrimidine derivatives, thymine and cytosine.

Under normal conditions Nucleic Acid is synthesised in the animal body from certain precursors, which are not at present definitely known. There is evidence, however, that the phosphoric acid is derived from the phosphorus of the phosphorised lipoids, while certain Amino-Acids are also essential to its synthesis.

In the absence of Water Soluble B factor from the food a condition of Nuclear starvation arises in all the organs. This nuclear starvation manifests itself clinically by a reduction in all the metabolic activities of the body and in the Central Nervous System by the occurrence of a functional paralysis.

Histologically, nuclear starvation is recognised by degenerative changes in the cell nuclei and by the storing up in the adrenals of one of the precursors of Nucleic Acid - phosphorised lipid.

Biochemically nuclear starvation is manifested by a reduction/



reduction in the Nucleic Acid content of the tissues, very marked in the liver, less marked in the brain. When Water Soluble B factor is administered to a bird with Beriberi, there is an end to nuclear starvation. The paralysis disappears, the lipoid is removed from the adrenals and the nucleic acid content of the brain returns almost to normal. The conclusion arrived at is that Water Soluble B factor is essential to the synthesis of Animal Nucleic Acid.

There is also some evidence to show that the Water Soluble B factor content of the tissues is related quantitatively to the Nucleic Acid content of the tissues. Until, however, chemical science has isolated and determined the actual composition of Water Soluble B factor, it will not be possible definitely to determine its exact relationship to the Nucleic Acid molecule,-to determine in fact whether Water Soluble B factor forms a chemical constituent or merely an adsorption compound of Nucleic Acid.





## DESCRIPTION OF PLATES.

The figures are grouped together to illustrate the main histological changes met with in the organs of Man and birds suffering from Beriberi.

## PLATE 1.

- Fig. 1 Dorsal Cord of Fowl suffering from Beriberi; portion of Anterior horn & white matter, showing a few scattered fibres with degeneration. Marchi. x 50.
- Fig. 2 Anterior horn of Cervical cord of Pigeon with Beriberi. Absence of glia cell reaction. Haematoxylin and eosin. x 300.
- Fig. 3 Anterior horn cells of Cervical cord of Pigeon with Beriberi. Chromatolysis. Nissl's stain. x 1000.
- Fig. 4 Purkinje cell of Cerebellum of Man with Beriberi; commencing Chromatolysis. Nissl's stain. x 1000.
- Fig. 5 Anterior horn cell of Cervical Cord of Man with Beriberi. Chromatolysis & Nuclear degeneration x 1000.
- Fig. 6 Longitudinal Section of Sciatic Nerve of fowl with Beriberi, showing "frothy" appearance of myelin sheaths. Heidenhain's Iron-haematoxylin. x 1000.

## PLATE 2.

- Fig. 7 Transverse Section of Sciatic Nerve of fowl with Beriberi. In a few fibres the axis cylinder has broken down. Haematoxylin & acid fuchsin. x 1000.
- Fig. 8 Adrenal of normal pigeon. Medullary areas dark, cortical areas light. Haematoxylin & eosin. x 300.
- Figs 9&10 Adrenals of pigeons with Beriberi. The cortical areas have increased in extent. Haematoxylin & eosin x 300
- Fig. 11 Adrenal of pigeon with Beriberi. Cortical cells loaded with lipoid. Scharlach R & Haematoxylin. x 300.
- Fig. 12 Adrenal of pigeon with Beriberi. Bird killed six hours after the administration of Water Soluble B factor. Disappearance of lipoid. Scharlach R and Haematoxylin. x 300.

## PLATE 3.

- Fig. 13 Adrenal from human case of Beriberi; no increase in fibrous tissue. Van Gieson's stain. x 50.
- Fig. 14 Adrenal from human case of Beriberi; nuclear degeneration. Heidenhain's iron-haematoxylin. x 300.
- Fig. 15 Adrenal from human case of Beriberi; Medullary cells contain lipoid. Sudan III & Haematoxylin. x 300
- Fig. 16 Adrenal from human case of Beriberi - cortex and medulla both contain lipoid. Sudan III and Haematoxylin. x 50.
- Fig. 17 Adrenal from human case of Beriberi. medulla contains lipoid. Osmic Acid. x 50.
- Fig. 18 Adrenal from human case of Beriberi - cortex and Medulla both contain lipoid. Scharlach R & Haematoxylin. x 50.

## PLATE 4.

- Fig. 19 Adrenal from human case of Beriberi - Medullary cells contain lipoid. Scharlach R. & Haematoxylin, x 300.
- Fig. 20 Adrenal from human case of Beriberi. Cortical cells with lipoid. Scharlach R. & Haematoxylin, x 300
- Fig. 21 Thyroid from normal fowl. Haematoxylin & Eosin. x 50.
- Fig. 22 Thyroid from pigeon with Beriberi. Atrophy and conversion into a parathyroid-like tissue. Haematoxylin & Eosin. x 50.
- Fig. 23 Thyroid from fowl with Beriberi. Slight atrophy Van Gieson's stain x 300.
- Fig. 24 Thyroid from human case of Beriberi. Heidenhain's iron-haematoxylin. x 50.

## PLATE 5

- Fig. 25 Thyroid from human case of Beriberi. slight atrophy. Heidenhain's iron-haematoxylin. x 300.
- Fig. 26 Spleen from normal pigeon. Haematoxylin & eosin. x 50.



- Fig. 27 Spleen from pigeon with Beriberi. Atrophy & pigmentation. Van Gieson's stain. x 50.
- Fig. 28 Spleen from fowl with acute inanition. Atrophy. Haematoxylin & Eosin. x 50.
- Fig. 29 Spleen from human case of Beriberi. slight atrophy. Haematoxylin & Eosin. x 50.
- Fig. 30 Testicle from normal pigeon. Scharlach R. & Haematoxylin. x50.

## PLATE 6.

- Fig. 31 Testicle from pigeon with Beriberi. Atrophy of tubules. Haematoxylin & Eosin. x 50.
- Fig. 32 Testicle from fowl with acute inanition Van Gieson's stain. x 50.
- Fig. 33 Testicle from normal pigeon with active spermatogenesis. Haematoxylin & Eosin. x 300.
- Fig. 34 Testicle from pigeon with Beriberi. Haematoxylin & Eosin. x 300.
- Fig. 35 Testicle from fowl with acute inanition. Haematoxylin & Eosin. x 300.
- Fig. 36 Kidney from normal pigeon. Haematoxylin & Eosin. x 50.

## PLATE 7.

- Fig. 37 Kidney from pigeon with Beriberi. congestion and degeneration in the convoluted tubules. Haematoxylin & Eosin. x 50.
- Fig. 38 Kidney from pigeon with Beriberi. congestion of glomeruli. Haematoxylin & Eosin. x 300.
- Fig. 39 Kidney from fowl with Beriberi. cloudy swelling of the convoluted tubules. Haematoxylin & Eosin. x 300.
- Fig. 40 Heart from fowl with Beriberi - slight atrophy. Haematoxylin & Eosin. x 50.
- Figs. 41 & 42 Heart from human case of Beriberi. atrophy and fragmentation of the fibres. Haematoxylin & Eosin. x 50 : x 300.

## PLATE 8.

- Figs 43 & 44 Heart Muscle from human case of Beriberi.  
Fatty degeneration. Sudan III & Haematoxylin.  
x 50 : x 300.
- Fig. 45 Radial Artery from human case of Beriberi.  
Fragmentation of Muscular fibres. Haematoxylin  
& Eosin. x 300.
- Fig. 46 Glandular stomach of normal fowl. Haematoxylin  
& Eosin. x 300.
- Fig. 47 Glandular stomach of fowl with Beriberi.  
Degeneration of Glandular cells.  
Haematoxylin & Eosin. x 300.
- Fig. 48 Liver of normal fowl. Haematoxylin & Eosin. x 50.

## PLATE 9.

- Fig. 49 Liver of fowl with Beriberi. Congestion and  
cloudy swelling. Haematoxylin & Eosin. x 50.
- Fig. 50 Liver of fowl with Beriberi. Nuclear degeneration.  
Haematoxylin & Eosin. x 300.
- Fig. 51 Liver of fowl with acute inanition. Parenchymatous  
degeneration. Haematoxylin & Eosin. x 300.
- Figs. 52 & 53 Liver of fowl with Beriberi. Fatty degeneration.  
Sudan III and Haematoxylin. x 50 : x 300.
- Fig. 54 Liver of human case of Beriberi. Parenchymatous  
degeneration. Haematoxylin & Eosin. x 300.





Fig. 1



Fig. 2



Fig. 3



Fig. 4



Fig. 5



Fig. 6



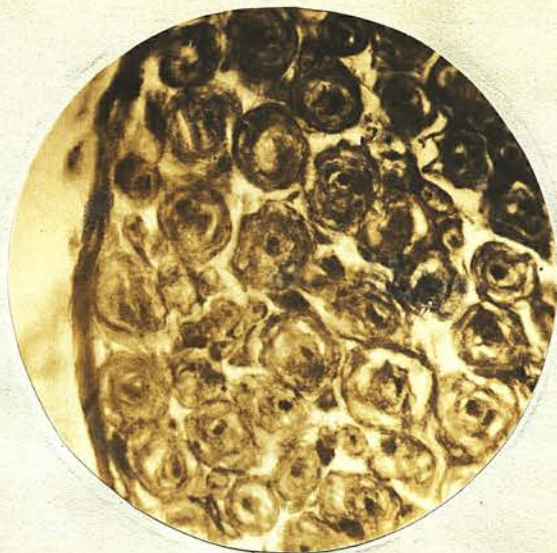


Fig. 7



Fig. 8



Fig. 9



Fig. 10

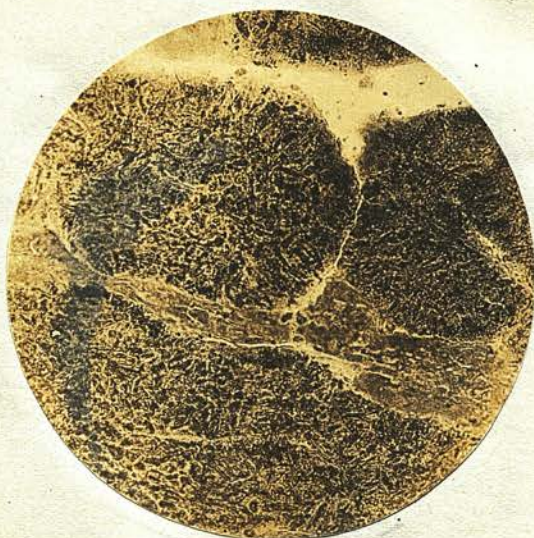


Fig. 11



Fig. 12





Fig. 13



Fig. 14



Fig. 15



Fig. 16



Fig. 17



Fig. 18





Fig. 19

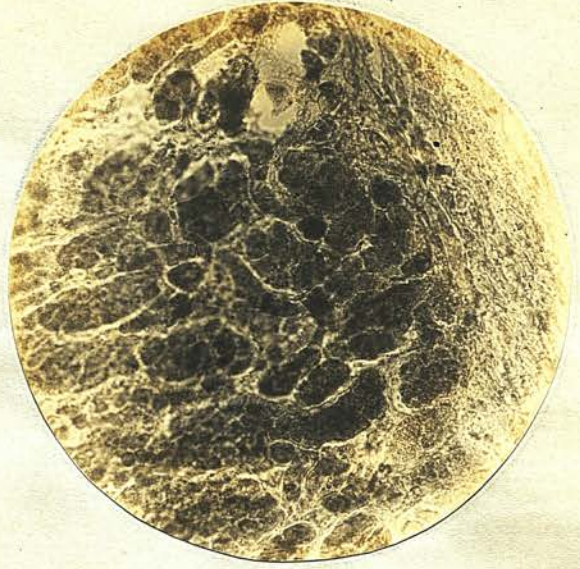


Fig. 20



Fig. 21



Fig. 22



Fig. 23

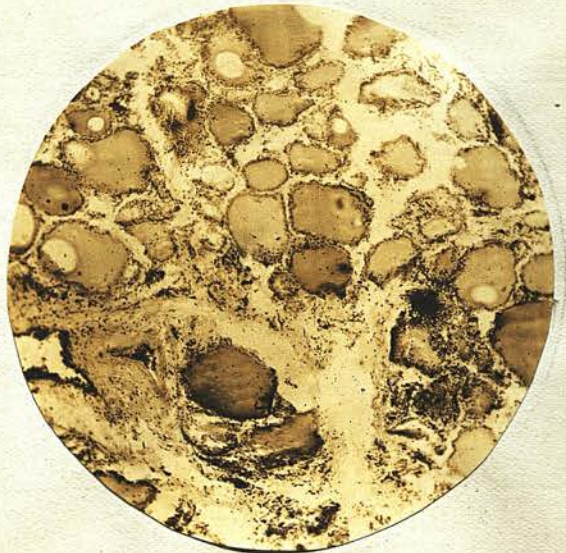


Fig. 24



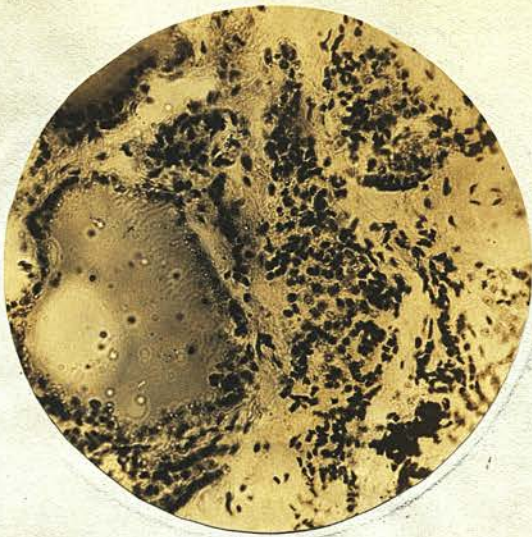


Fig. 25



Fig. 26



Fig. 27



Fig. 28

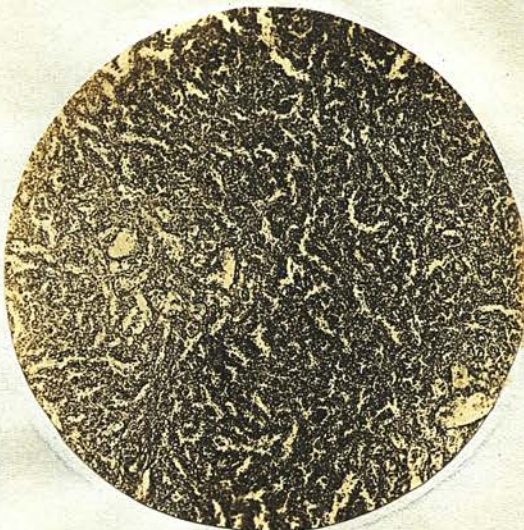


Fig. 29



Fig. 30





Fig. 31



Fig. 32



Fig. 33



Fig. 34



Fig. 35



Fig. 36





Fig. 37



Fig. 38



Fig. 39



Fig. 40



Fig. 41



Fig. 42





Fig. 43



Fig. 44



Fig. 45

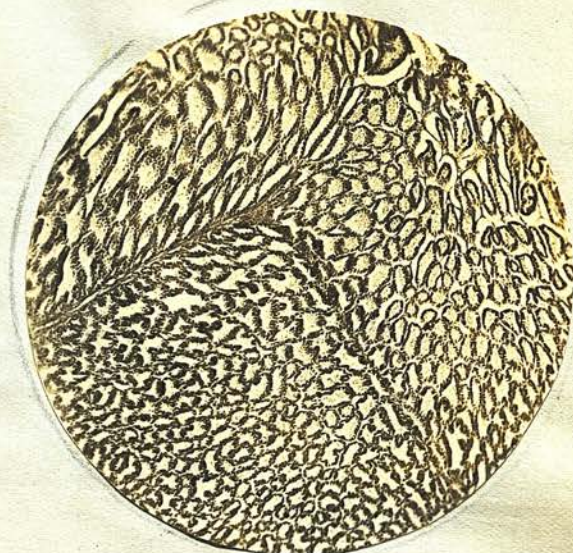


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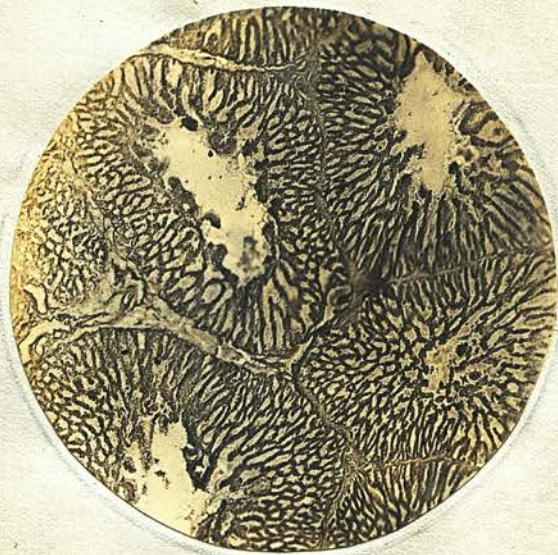


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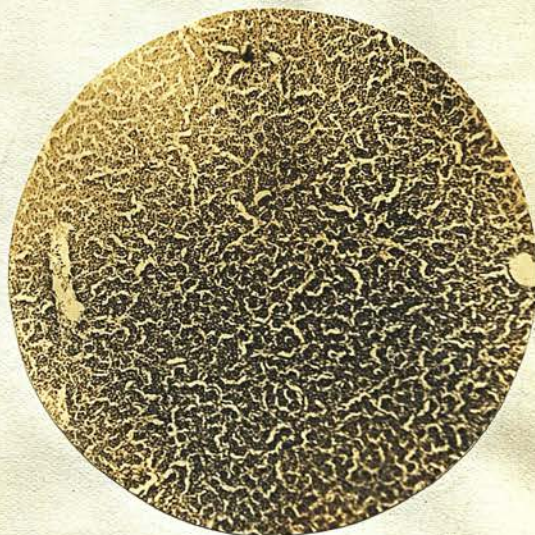






Fig. 49



Fig. 50



Fig. 51



Fig. 52

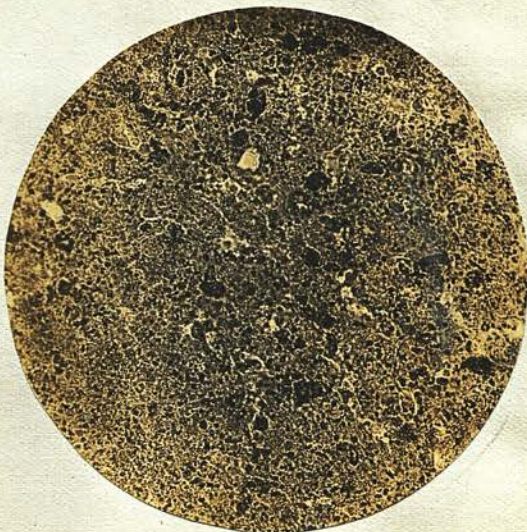


Fig. 53

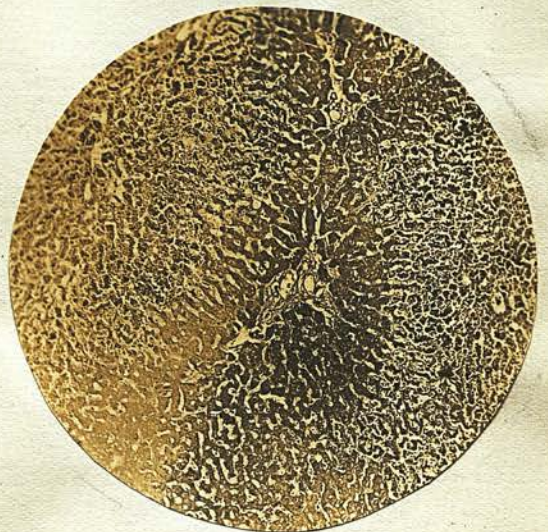


Fig. 54